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CEA-Related CAMs

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Abstract The carcinoembryonic antigen (CEA) family comprises a large number of cellular surface molecules, the CEA-related cell adhesion molecules (CEACAMs), which belong to the Ig superfamily. CEACAMs exhibit a complex expression pattern in normal and malignant tissues. The majority of the CEACAMs are cellular adhesion molecules that are involved in a great variety of distinct cellular processes, for example in the integration of cellular responses through homo- and heterophilic adhesion and interaction with a broad selection of signal regulatory proteins, i.e., integrins or cytoskeletal components and tyrosine kinases. Moreover, expression of CEACAMs affects tumor growth, angiogenesis, cellular differentiation, immune responses, and they serve as receptors for commensal and pathogenic microbes. Recently, new insights into CEACAM structure and function became available, providing further elucidation of their kaleidoscopic functions.

Keywords CEA-related cellular adhesion molecules · Immunoglobulin superfamily member · Adhesion · Signal transduction · Microbial receptor

1 Introduction

Carcinoembryonic antigen (CEA)-related cellular adhesion molecules (CEACAMs; pronounced C-CAMs) belong to the Ig superfamily of cellular surface molecules. CEA was discovered by Gold and Freedman as a tumor-associated antigen in human colorectal carcinoma (Gold and Freedman 1965). Originally, CEA was considered an oncofetal protein that is re-expressed in adult tissues only during carcinogenesis. Later, it became evident that CEA is expressed both in embryonic and healthy adult tissues. CEA levels in serum are elevated during the progression of various malignant diseases, namely cancer of the colon, breast, and lung. CEA serves as a clinical tumor marker and is of important prognostic relevance in the evaluation of progressive colonic carcinoma: increasing serum levels indicate recurrence and residual disease (Thompson 1991). After the cDNA of CEA had been cloned and characterized independently by various groups, CEA could be assigned to the Ig superfamily by sequence homology studies (Beauchemin et al. 1987; Oikawa et al. 1987; Paxton 1987; Zimmermann et al. 1987; Schrewe et al. 1990).

Members of the Ig superfamily of cell surface proteins do not only exert immune regulatory functions, but they also mediate cellular recognition and adhesion (Williams and Barclay 1988; Brümmendorf and Lemmon 2001; Juliano 2002). In fact, out of 26,383 human genes with a known or predicted function, 577 (1.9%) encode proteins that are involved in cell adhesion, and 264 (0.9%) encode immunoglobulins. Proteins containing Ig domains are among the most frequently expressed in the human genome, with 930 Ig domains contained in 381 Ig family members (Venter et al. 2001). Ig domains can form rods when arranged in series, and their ability to specifically recognize self or non-self proteins, or participate in *cis*- and *trans*-interactions,

and regulate signaling and adhesion, make them principal players in orchestrating complex cellular responses. Moreover, alternative splicing and extensive N-linked glycosylation are used as a versatile tool to generate different isoforms, creating an even broader platform for recognition processes and signal integration.

The CEA-related adhesion molecules were discovered upon their cross-reactivity with CEA antisera and based on sequence homology studies after cloning of novel cDNAs. The first CEACAMs to be identified were the NCAs (non-specific cross-reacting antigens), independently described by the group of von Kleist et al. (1972) and Mach et al. (1972). Biliary glycoprotein (BGP) was originally described by Svenberg (1976) as being expressed in normal human gall bladder mucosa and at the bile canalicular surface of hepatocytes. BGP, also referred to as NCA-160, C-CAM (cell–cell adhesion molecule), C-CAM105, H4A, or pp120, is now called CEACAM1. In the course of the discovery of new genes and proteins that belong to the CEA gene or protein family, their nomenclature became quite inconsistent. Novel proteins were also named CGMs (CEA-gene family members), and well-conserved homologs to CEA-like genes in rodents have been assigned a variety of names. To date, 29 CEA-like genes are known in the human genome, 19 in the murine genome and 7 in the rat. According to their structural similarities, they were classified into three subgroups: CEA-like-genes, the PSG-subgroup (pregnancy-specific glycoproteins), and the pseudogenes. An overview of the CEA-family subgroup is shown in Fig. 1.

In this review, the nomenclature used is based on its most recent revision (Beauchemin et al. 1999; http://cea.klinikum.uni-muenchen.de). For common historical names and abbreviations, the reader is referred to Fig. 1 and Beauchemin et al. (1999).

All members of the CEA-subgroup share the following common characteristics. They are members of the Ig superfamily, they are membrane bound, and they are heavily glycosylated adhesion molecules (Odin et al 1986; Beauchemin et al. 1999). CEACAMs are multifunctional. Their biological functions comprise cellular homophilic and heterophilic adhesion, suppression of tumor cell growth, regulation of cell growth and differentiation, binding of a variety of different pathogens, and acting as growth factors for endothelial cells, as well as binding to a variety of intracellular adaptors in central signal transduction cascades.

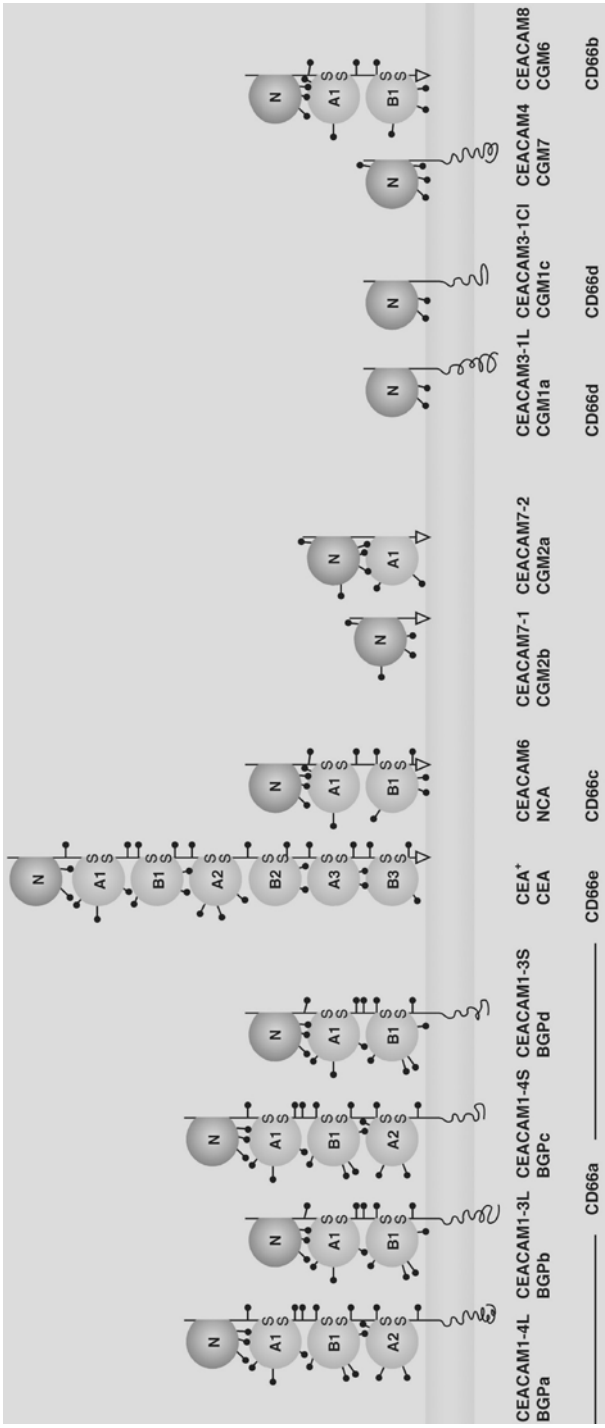

Fig. 1. Schematic presentation and nomenclature of the CEA-subgroup. The immunoglobulin variable-like domains are shown in *dark gray*. Note that they do not contain disulfide linkages as in the immunoglobulin constant-like domains. Positions of N-linked glycosylation sites are represented by *lollipops*. Linkage to the plasma membrane by glycosylphosphatidylinositol anchors is indicated by *arrows*. Different splice variants with either a long (*L*) or short (*S*) cytoplasmic domain are indicated. Names of the individual family members according to the latest revision of the CEA gene family nomenclature are shown in the *upper line* (Beauchemin 1999). In the *second line*, common historical names of the proteins and different splice variants are used. In the *third line*, CEACAMs are grouped according to their designation as leukocyte differentiation antigens. [+], For historical reasons, the product of the *CEACAM5* gene, the CEA protein, is referred to as CEA. (Reproduced from Hammarström and Baranov 2001, with permission from Elsevier Science)

2
Genomic Organization and Regulation of Transcription

2.1
Gene Structure

The human CEA gene family comprises 29 genes that have been divided into three subgroups according to their DNA and amino acid sequence and structural homology: (1) 12 CEA genes, 7 of which are expressed (CEACAM1-12); (2) 11 PSG genes, 9 of which are expressed, and (3) a group of 6 pseudogenes (CEACAM13 through 18), for which no cDNA has yet been identified.

The CEA gene family emerged from a common ancestor gene that initially gave rise to block of three genes with the same transcriptional orientation. Repeated duplication and inversion of these gene blocks produced the CEA family gene cluster, located on the long arm of human chromosome 19 (q13.2, between CYP2A and D19S15). Within its expanse of 1.8 Mb, the CEA- and PSG-subgroup genes are organized in two clusters of 250 and 850 kb, respectively. The proximal half includes members of the CEA subgroup, and the distal half comprises the 11 PSG-subgroup genes with the pseudogenes interspersed. The two clusters are interrupted by a 700-kb region encoding 5 nonrelated proteins (Thompson et al. 1989; Kahn et al. 1992; Kahn et al. 1994; Olsen et al. 1994; Teglund et al. 1994). CEACAM1 is the most ancestral gene of the CEA-gene family. Whereas there is only one gene encoding the human and rat CEACAM1-protein, there are two closely related genes in the mouse (*Ceacam1* and *Ceacam2*).

The aminoterminal Ig variable-type domain (IgV) is highly homologous to that of other CEA-gene family members. At the same time, the IgV-like domain is more susceptible to variations in its amino acid sequence than the Ig constant-like (IgC) C2-like domains. CEACAMs are undergoing a dynamic evolution. All members of the CEA gene family share a common intron–exon organization. They contain an exon encoding 5′ UTR (untranslated region) and part of the leader peptide, followed by an exon encoding the rest of the signal-peptide (34 aa) for membrane targeting and the N-terminal IgV-like domain (108 aa). It is followed by exons encoding the IgC set-like domains of either the A- (93 aa) or the B-type (85 aa) that are arranged in A+B pairs. Each of these exons encodes a complete Ig domain. Downstream of this region are exons encoding the C-terminal portion and the 3′ UTR.

The number of Ig domains in CEACAM1 varies from one to four domains as a result of alternative mRNA splicing. This also gives rise to two isoforms that differ in the length of their cytoplasmic domains, a long form, referred to as CEACAM1-L, composed of 70–73 amino acids, or a short form, referred to as CEACAM1-S, and comprising 10–12 amino acids (McCuaig et al. 1992; Edlund et al. 1993; Najjar et al. 1993; McCuaig et al. 1993). In contrast

to the human *CEACAM1* gene, there are two allelic variants in the mouse and in the rat, *Ceacam1a* and *Ceacam1b* (Edlund et al. 1993; McCuaig et al. 1993). These allelic variants exhibit their greatest genetic variation within the N-terminal domain. The most important isoforms of murine CEACAM1 contain either two or four extracellular Ig-like domains (the N-teminal domain and either the A2 or A1 and the B1 domain (Beauchemin 1999).

So far, the splice variants of human CEACAM1 are the best characterized (Barnett et al. 1989; Barnett et al. 1993). To date, there are at least 12 known splice variants. The most prominent isoforms are CEACAM1-4L and CEACAM1-4S, each containing four extracellular Ig-like domains and either a long or a short cytoplasmic tail.

2.2 Regulation of Transcription

The promoters of the CEA gene family member genes exhibit features of housekeeping genes. They lack classical TATA or CCAAT boxes, and they contain GC-rich regions and Sp1 sites. In the human and rat *CEACAM1*-gene, binding sites for activator protein (AP)1 and AP2-like factors, USF (upstream stimulatory factor), and HNF-4 (hepatic nuclear factor-4) could be identified (Hauck et al. 1994; Najjar et al. 1996).

However, in contrast to classical housekeeping genes, *CEACAM1*-genes are subjected to cell- and tissue-specific developmental and differential regulation (Schrewe et al. 1990; Hauck et al. 1991) Furthermore, they respond to changes in the hormonal status in certain tissues and cell lines: their expression can be induced by cyclic adenosine monophosphate (cAMP), retinoids, androgens, estrogens, glucocorticoids, or insulin (Svalander et al. 1990; Botling et al. 1995; Hsieh et al. 1995; Daniels et al. 1996; Najjar et al. 1996; Makarovskiy et al. 1999; Phan et al. 2001). CEA gene family members respond to inflammatory stimuli as well, such as interferons, tumor necrosis factors, and interleukins (Takahashi et al. 1993; Chen et al. 1996; Kammerer et al. 1998).

3 Three-Dimensional Structure

Modeling of the three-dimensional structure of members of the CEA gene family revealed the characteristic Ig fold (Bates et al. 1992; Boehm 1996; Tan et al. 2002). All human CEA-subgroup members contain one IgV-like domain and zero to six C-like domains. They are linked to the membrane by a transmembrane anchor, followed by a long or short cytoplasmic tail, a glycosylphosphatidylinositol (GPI)-anchor or, in case of the soluble PSG-subgroup, a short hydrophilic tail. In contrast to the human CEA family pro-

teins, rodent CEACAMs may contain several IgV-like domains (Rudert et al. 1992). GPI-linked proteins of the CEA family are not expressed in rodents. CEA-related adhesion molecules are heavily glycosylated; they contain zero to six Asn-X-Ser/Thr-motifs for potential N-linked glycosylation per Ig domain. The glycosylation of CEACAMs comprises high-mannose and complex type oligosaccharides like lactosaminoglycans type I and type II chains that are terminated by fucosyl- and sialyl-residues (Odin et al. 1986; Mahrenholz et al. 1993; Stocks et al. 1993; Fukushima et al. 1995; Kannicht et al. 1999; Sanders and Kerr 1999). Type I and type II lactosaminoglycans constitute the Lewis blood group antigens. Prominent carbohydrate moieties of this group on CEACAM1 and CEA are Lewis^x and sialyl-Lewis^x antigens (Sanders and Kerr 1999). On CEACAM1, the presence of high-mannose residues seems to be restricted to its membrane proximal A2-domain (Mahrenholz et al. 1993; see also Fig. 7).

Modeling of the CEACAMs' three-dimensional structures, i.e., CEACAM1 and CEA, has been performed on the basis of structural comparison with other representative members of the Ig superfamily, namely CD2, CD4, CD8, and the Bence-Jones protein REI (Tan et al. 2002 and references therein). In a recent structural analysis performed with a soluble murine CEACAM1a, splice variant comprising the N-terminal IgV-like domain and the membrane proximal IgC-like domain (CEACAM1a[1,4]), the structure of the IgC-like domains in CEACAMs was found to resemble the I-like Ig fold rather than the C2-fold. Referring to data obtained earlier after structural modeling of human CEA, it can be hypothesized due to high inter-species structural conservation of the CEACAMs, that the A-type domains belong to the I1 set and the B-type domains belong to the I2 set fold. The variable-like N-terminal domains of the CEACAMs lack the characteristic disulfide linkage between the beta strands B and F but nonetheless meet the criteria for a V set Ig-like fold (Bates et al. 1992; Harpaz and Chothia 1994; Chothia et al. 1998; Wang and Springer 1998; Tan et al. 2002).

CEACAMs exhibit unique structural features when compared to other members of the Ig superfamily. Structurally, the N-terminal domain of CEACAM1 and other members of the CEA family is exceptional in that its CC′ loop is convoluted and folds back onto the A′GFCC′C″ β-sheets, called CFG face hereafter (Fig. 2). Moreover, within the CEA family, the ABED face in the N-terminal domain is much more conserved than the CFG face. The CFG faces of variable-like domains are frequently used in cell surface recognition (Stuart et al. 1995; Wang and Springer 1998). This has been demonstrated for viral binding to host cell receptors, as specific sequences within this region confer the specificity for a cognate receptor. The variability in this region of the CEACAMs determines their distinctive binding properties in homophilic and heterophilic adhesion but also in their binding to extracellular ligands and as targets for microbes and viruses. Regarding the role of the N-terminal domain in *trans*-homophilic adhesion among CEACAM1

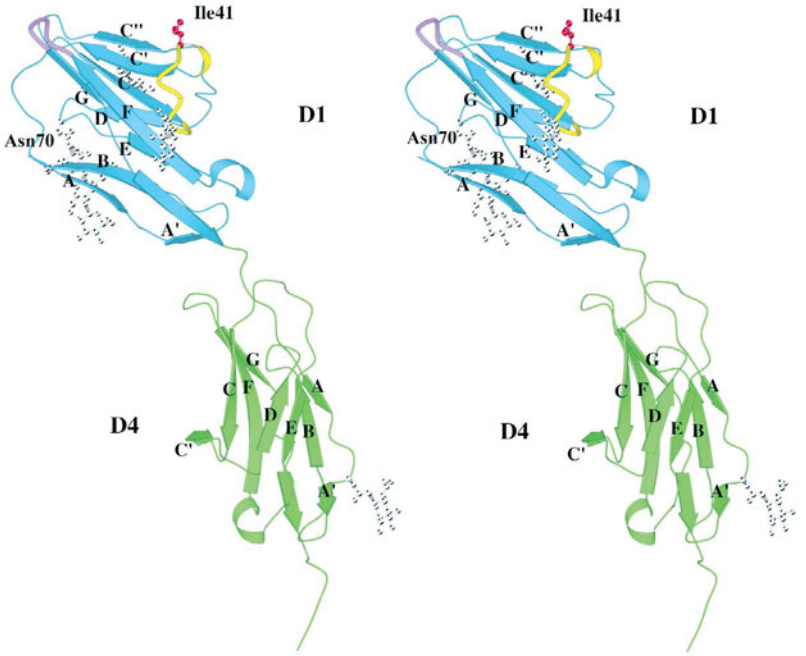

Fig. 2. Stereo view of the ribbon drawing of soluble murine CEACAM1a[1,4], which contains the amino-terminal IgV-like (*D1*) and the membrane proximal IgC-like domain (*D4*). The *ribbon drawing* reveals the anti-parallel β-sheets that constitute the immunoglobulin fold. The uniquely convoluted CC′ loop in the N-terminal domain which is involved in molecular recognition processes by CEACAMs, such as binding murine hepatitis virus and other ligands (see there in this review), is highlighted in *yellow*. The predicted key virus-binding residue Ile41 on the CC′ loop is shown in *red* in *ball-and-stick* representation. The N-linked glycan at Asn70 in the D1 domain that is conserved through the whole CEA family is labeled accordingly. (Reproduced from Tan et al. 2002, with permission)

and CEA, for example, it has been shown that interactions between the CGF face, more specifically the CC′ loop, are essential to promote homophilic binding (Teixeira et al. 1994; Watt et al. 2001).

This review will focus on functions of CEACAM1, as they are known to date. The data summarized here have been collected from various experimental in vitro-model systems, as well as from in vivo studies in mouse and rat. Additionally, clinical data are included.

4
Expression Pattern

4.1
Expression of CEACAMs in Healthy Adult Tissues

CEACAMs display a very heterogeneous expression pattern. Crossreactivity between several anti-CEACAM antibodies hampered precise characterization of their individual expression pattern until more specific antibodies became available. Summaries about reactivity of certain anti-CEACAM antibodies and antisera can be found in Nap et al. (1992) Schölzel et al. (2000) and Watt et al. (2001).

To date, the expression patterns of CEA (encoded by the *CEACAM5* gene), CEACAM1, CEACAM6, and CEACAM7 have been characterized best (Prall et al. 1996; Stanners 1998). Recently, more detailed information about the expression pattern of CEACAM6 and CEACAM7 have become available (Schölzel et al. 2000). However, there is relatively little information available on the expression pattern of CEACAM3, CEACAM4, and CEACAM8. Generally, CEACAMs are subjected to developmental and differential regulation in a spatiotemporal fashion.

Despite their different sites of expression, CEACAMs can be categorized into four major groups:

1. Selective epithelial expression pattern: CEA, CEACAM7—with pronounced apical expression
2. Expression on granulocytes: CEACAM3,8
3. Broad expression: CEACAM1,6
4. Predominant expression pattern in the syncytiotrophoblast: CEACAM-ps1–11 (formerly PSGs,)

CEACAM1, CEACAM3, and CEACAM4 contain a hydrophobic transmembrane domain followed by a long or short cytoplasmic domain, whereas CEACAM2, CEA, CEACAM6, and CEACAM7 are attached to the membrane via a glycosylphosphatidyl inositol anchor. CEA family members with GPI anchors are not expressed in rodents. The expression pattern of CEACAMs in mouse and human is very similar. However, in contrast to human, there are two CEACAM1 genes in mice, *Ceacam1* and *Ceacam2,* that display slightly different expression patterns; whereas gene products of the *Ceacam1* gene are predominantly found in the majority of epithelia, leucocytes, and endothelia, *Ceacam2* is mainly expressed in spleen, kidney, and testes (Robitaille et al. 1999; Han et al. 2001).

CEACAM1 displays the broadest expression pattern among CEACAMs that is conserved in humans and rodents. It is expressed on various epithelia, such as esophagus (glandular epithelial cells), stomach (pyloric mucous

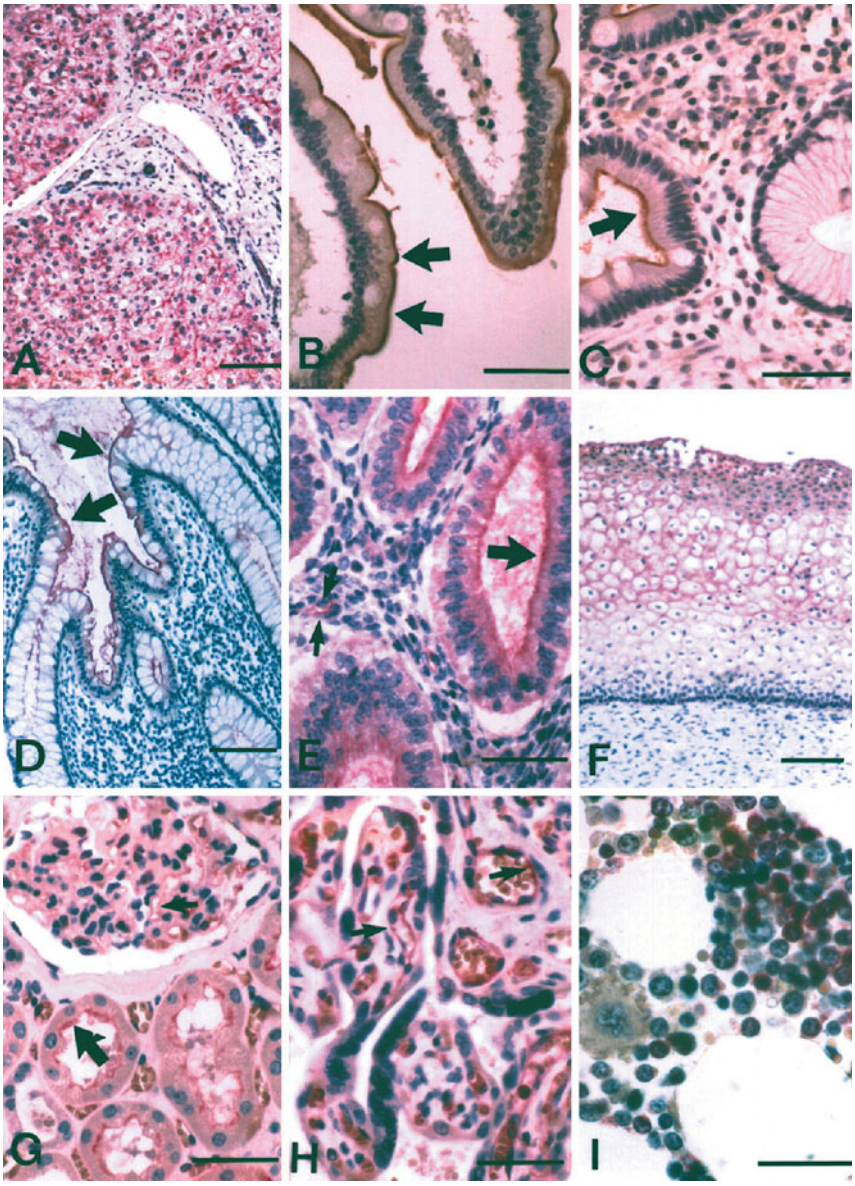

Fig. 3A–I. Expression pattern of human CEACAM1 in various human tissues in paraffin sections, as revealed by binding of the monoclonal anti-human CEACAM1-antibody 4D1/C2 (Drzeniek 1991) in: **A** liver, APAAP detection (alkaline phosphatase-anti alkaline phosphatase), **B** small intestine, ABC detection (avidin-biotin-complex), **C** glands from the gastroduodenal junction with enterocytes and mucosa glands, avidin biotin peroxidase complex (ABC) detection, **D** colon, alkaline phosphatase anti-alkaline phosphatase (APAAP) detection, **E** endometrium, APAAP detection, **F** uterine portio, APAAP detection, **G** kidney, APAAP detection, **H** placenta, APAAP detection, and **I** bone marrow,

cells, Brunner's gland cells), in epithelial cells of the duodenum, jejunum, and ileum; in colon (columnar epithelial cells, caveolated cells), pancreas (ductal epithelial cells), liver (bile canaliculi, bile duct epithelial cells), gall bladder (epithelia), in kidney epithelial cells in the proximal tubuli, the urinary bladder (transitional epithelial cells), prostate epithelial cells, cervix, squamous epithelial cells, endometrium, glandular epithelial cells, and in sweat and sebaceous glands (Fig. 3). Furthermore, CEACAM1 is expressed on granulocytes, leucocytes (T- and B-lymphocytes, monocytes), dendritic cells, and on endothelial cells in some organs (Hanenberg et al. 1994; Frängsmyr et al. 1995; Prall et al. 1996; Kammerer et al. 1998; Kammerer et al. 2001). Its two major isoforms, CEACAM1-L and CEACAM1-S, seem to be co-expressed in all tissues investigated so far (Baum et al. 1996; Turbide et al. 1997) with the exception of breast, endothelia, and T lymphocytes.

CEACAM6 also displays a fairly broad expression pattern, as it shares common sites of expression with CEACAM1: CEACAM6 is expressed in epithelia of different organs, and in granulocytes and monocytes (Kodera et al. 1993; Metze et al. 1996; Schölzel et al. 2000).

CEA exhibits a more restricted expression pattern in normal adult tissues. It is expressed on columnar epithelial cells in the colon, in mucous neck and goblet cells, in the pyrolic mucous cells of the stomach, and in squamous epithelial cells of the tongue, esophagus, prostate, and cervix as well as in secretory epithelia and duct cells of sweat glands. CEA expression has also been described on endothelia (Majuri et al. 1994). Interestingly, as shown in transgenic mouse models, the spatiotemporal expression pattern of CEA expressed under the transcriptional control of the *CEACAM5* promoter is preserved in transgenic animals (Eades-Perner et al. 1994). CEACAM7 is expressed in a pattern similar to CEA in colon, but is not found in granulocytes. CEACAM3 and CEACAM8 are expressed in granulocytes but have not been described in epithelial cells so far.

For the PSGs, the main expression site is the placenta, displaying high expression during the first trimester of pregnancy. Their expression is restricted to the syncytiotrophoblast (Rebstock et al. 1993; Zhou et al. 1997).

APAAP detection. CEACAM1 shows a pronounced apical location in B, C, D, E, and G (*large arrows*), and the staining of endothelia in E, G, and H (*small arrows*). *Bars*: A,D,F=100 μm; B, C, E, G, H=50 μm; I=25 μm. (Reproduced from Prall et al. 1996 with permission)

4.2
Expression of CEACAMs in Healthy Adult Colon

The expression of different members of the CEA-family has been extensively studied in the human colon, where they are a major component of the epithelial defense barrier, consisting of the glycocalyx and a supraepithelial mucus layer (Baranov et al. 1994; Frängsmyr et al. 1995; Frängsmyr et al. 1999; Hammarström and Baranov 2001). Four members of the CEA family

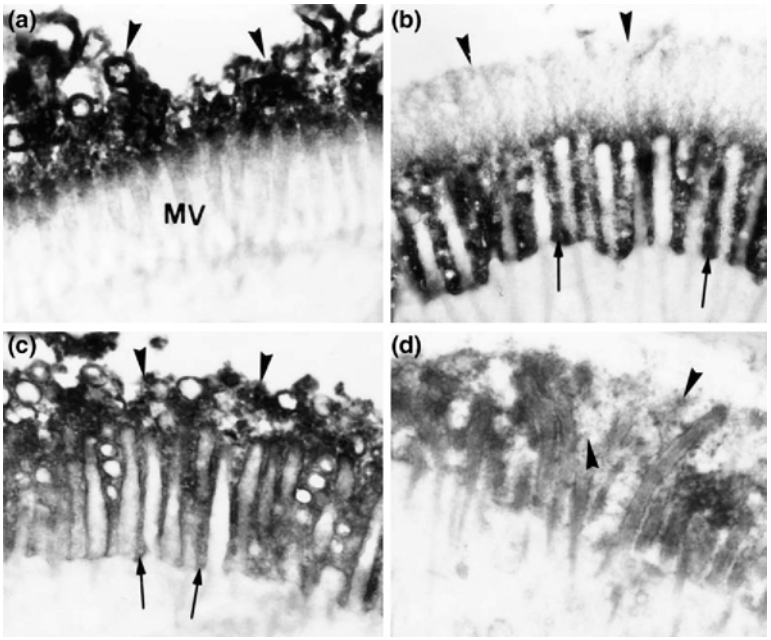

Fig. 4a–d. Immunoelectron microscopy of CEA, CEACAM1, CEACAM6, and CEACAM7 in normal human colon (indirect immunoperoxidase method). **a** Micrograph of the apical portion of a columnar cell. CEA-positive material is detected over the top of the microvilli (*MV*), and consists of thick long filaments and membrane vesicles (*arrowheads*). **b** Micrograph of the apical part of a mature columnar cell. Electron-dense CEACAM7-positive material is present between microvilli (*arrows*). Thin long matted filaments at the top of the microvilli are weakly stained. c Micrograph of the apical part of a mature columnar cell. CEACAM6-positive granular compact material, including membrane-bound vesicles, is seen between (*arrows*) and over the top of the microvilli (*arrowheads*). **d** Micrograph of the apical region of a mature columnar cell. A delicate CEACAM1-positive material that consists of very thin loose filaments is mainly located between the microvilli (*arrowheads*). Magnifications (a–d): ×20,000. The following murine monoclonal antibodies (mAb) were used: anti-CEA mAb Bu-103 (Baranov et al. 1994), anti-CEACAM6 mAbMox-36 (Baranov et al. 1994), anti-CEACAM1 mAb 4D1/C2 (Drzeniek et al. 1991), and anti-CEACAM7 mAb BAC2 (Frängsmyr et al. 1999). (Reproduced from Hammarström and Baranov 2001, with permission from Elsevier Science)

are expressed in the healthy colon, CEACAM1, CEA, CEACAM6, and CEACAM7. All four of these members of the CEA family are specifically localized on the apical surface of mature columnar epithelial cells (enterocytes), in microvesicles, filaments, and on highly differentiated epithelial cells at the crypt mouth. No expression can be detected on the basolateral side of the gut epithelium (Hansson et al. 1989; Frängsmyr et al. 1999). CEACAMs are a major component of the brush-border glycocalyx. However, regarding cellular differentiation, these CEACAMs display a compartmentalized expression pattern. Whereas CEA is exclusively located on the top of microvilli, and CEACAM7 is restricted to their lateral sides, CEACAM1 and CEACAM6 are expressed at the apical and lateral part of microvilli (Fig. 4). CEA and CEACAM6 are also expressed on goblet cells. Undifferentiated or maturing epithelial cells of mid and lower crypts are devoid of CEACAM1 and CEACAM7, and synthesize CEA and CEACAM6 only at low levels (Hammarström and Baranov 2001; Fig. 4).

4.3 Expression of CEACAMs During Gestation and Embryonic Development

Expression of CEA family members starts during the early fetal period and is maintained throughout life. Their expression can be detected within the first trimester of pregnancy in the embryo in humans and rodents and increases towards the end of embryonic development. Moreover, CEACAM1 and PSGs are expressed in the invasive trophoblast during gestation (Rebstock et al. 1993; Daniels et al. 1996; Bamberger et al. 2000).

CEA is present in normal human tissues in the fetus and in the adult in endoderm-derived tissues exclusively (Nap et al. 1988). The expression of CEA and other CEA-gene family members can be detected in early stages of pregnancy during the first trimester in human (Wagener et al. 1983; von Kleist et al. 1986). CEACAM1 expression also starts in the early phases of development and displays an interesting spatiotemporal regulation. In human and in mouse, it is expressed in the placenta on the maternal–fetal interface of the invasive extravillous trophoblast. CEACAM1 is also present on epithelial cells of pregnancy epithelium, but the decidua is devoid of CEACAM1. As in the human, CEACAM1 is also expressed during early stages in rodent development. Its expression can be detected as early as day 7.5 in the postimplantation conceptus, namely in the visceral yolk sac and in the invasive syncytiotrophoblast (Huang et al. 1990; Daniels et al. 1996; Sawa et al. 1997; Bamberger et al. 2000). Furthermore, CEACAM1 is expressed on maternal blood vessels, and a strong reactivity for anti-CEACAM1-specific antibodies is also found on embryonic capillaries. In the mouse embryo, the first sites of CEACAM1 expression are the primitive gut during early morphogenesis of the surface ectoderm, and in areas of epithelial–mesenchymal interactions, i.e., in the dermis, meninges, lung, kidney, salivary glands, and

the pancreas. CEACAM1 is also detected during myogenesis and odontogenesis (Rass et al. 1994; Lüning et al. 1995; Daniels et al. 1996).

4.4 Dysregulation of the Expression of CEACAMs in Human Tumors

CEACAMs are expressed in various tumors of epithelial origin (colorectal carcinoma, lung adenocarcinoma, mucinous ovarian carcinoma, endometrial adenocarcinoma). Overall, the expression of CEA is more restricted in tumors than CEACAM1 and CEACAM6, which are also dysregulated in leukemias, hepatocellular carcinoma, and melanoma (summarized in Table 1).

In contrast to other CEA family members that are down- or upregulated in human tumors, CEACAM1 displays a rather conflicting expression pattern. In human colon, prostate, and hepatocellular carcinoma, for example, CEACAM1 is downregulated when compared to healthy tissue specimens. However, CEACAM1 is upregulated in gastric and squamous lung cell carcinomas or in malignant melanoma. In cases of CEACAM1 downregulation during early tumorigenesis, the loss of CEACAM1 expression is accompanied by a dramatic alteration in tissue architecture, caused by changes in cell polarity and adhesion. The effects observed after downregulation of CEACAM1 expression are phenotypically quite similar to those observed after the mutation of cadherins or loss of their expression during malignant progression (Birchmeier and Behrens 1994; Bracke et al. 1996). The impact of CEACAM1 expression on tissue architecture has been clearly demonstrated for hepatic, colorectal, and prostate carcinomas (Hixson et al. 1985; Nollau et al. 1997; Busch et al. 2002). In this context, it is noteworthy that restoration of CEACAM1-4S expression in mammary carcinoma cells induces lumen formation and reversion of the tumorigenic phenotype in a cell culture model (Kirshner et al. 2003a). In a marked contrast to these observations, CEACAM1 is re-expressed in invasive melanoma and lung adenocarcinoma (Laack et al. 2002; Thies et al. 2002). In the case of malignant melanoma, CEACAM1 expression was especially found at the invasive front of the tumors and maintained in their metastatic lesions. Moreover, CEACAM1 was identified as a *cis*-binding partner of integrin $\alpha_V\beta_3$ in a variety of epithelial cell lines, endothelial cells, and malignant melanoma (Brümmer et al. 2001). Hence, it is possible that *cis*-interaction of CEACAM1 with integrins can promote invasion. Downregulation of CEACAM1 during the progression of colonic carcinoma is an early event in tumorigenesis, indicating that loss of its expression is the result of a genetically based alteration (Neumaier et al. 1993). However, with regards to invasion, its invasive potential seems in part to depend on its binding partners in *cis* and its phosphorylation status (A.K. Horst, C. Wagener, and N. Beauchemin, manuscript in preparation).

Table 1. Expression of CEA family members in human tumors (Hammarström 1999). Overview of dysregulated expression of CEA gene family proteins in a variety of human tumors

Type of tumor	CEA	CEACAM6 (NCA)	CEACAM1 (BGP)	CEACAM8 (CGM6)	CEACAM3 (CGM1)	CEACAM7 (CGM2)	CEACAM4 CGM7	PSGs	Reference(s)
Epithelial									
Colorectal carcinoma	+	+↑	+↓			+↓			Thompson, 1994; Baranov, 1994; Kim et al. 1992; Jothy et al. 1993; Neumaier, 1993; Thompson et al. 1993; Cournoyer et al. 1988; Sheahan et al. 1990; Tsutsumi et al. 1990; Shi et al. 1994; Thompson et al. 1997
Gastric carcinoma	+↑	+↑	+↑			+↑			Kinugasa et al. 1998; Shi et al. 1994; Kodera et al. 1993
Lung adenocarcinoma	+	+	+						Kim et al. 1992; Cournoyer et al. 1988; Shi et al. 1994; Robbins et al. 1993; Laack et al. 2002; Sienel et al. 2003
Lung squamous cell carcinoma	−		+↑						Tsutsumi et al. 1990; Ohwada et al. 1994
Breast carcinomas	(+)	+	+			−			Thompson, 1994; Shi et al. 1994; Robbins et al. 1993; Cournoyer et al. 1988; Thompson et al. 1993; Bamberger et al. 2002
Pancreatic carcinoma	+								Shi et al. 1994
Gallbladder carcinoma	+								Shi et al. 1994
Mucinous ovarian carcinoma	+	+	(+)	−	−	+	−	−	Thompson 1994
Serous ovarian carcinoma	(+)	(+)	(+)	−	−			−	Thompson et al. 1993
Endometrial adenocarcinoma	+	+	+	−	−		−		Thompson et al. 1993
Hepatocellular carcinoma	−		+↓						Shi et al. 1994; Hinoda et al. 1990; Tanaka et al. 1997
Thyroid carcinoma	−								Shi et al. 1994
Nasopharyngeal carcinoma	−								Shi et al. 1994
Prostate			+↓						Busch et al. 2002

Table 1. (continued)

Type of tumor	CEA	CEACAM6 (NCA)	CEACAM1 (BGP)	CEACAM8 (CGM6)	CEACAM3 (CGM1)	CEACAM7 (CGM2)	CEACAM4 CGM7	PSGs	Reference(s)
Other									
Malignant mesothelioma	–								Dejmek and Hjerpe 1994
Small cell lung carcinoma	+		–						Kim et al. 1992; Ohwada et al. 1994
Acute lymphoblastic leukaemia	–	+	(+)		–		–		Hanenberg et al. 1994
Multiple myeloma			+						Satoh et al. 2002
Melanoma	–		+						Shi et al. 1994; Thies et al. 2002
Different sarcoma	–								Shi et al. 1994
Hydatidiform mole								+	Leslie et al. 1990
Choriocarcinoma								+	Leslie et al. 1990

Note that the expression of certain CEACAMs has not been determined completely in all malignant tissues summarized here. Also see Hammarström (1999) and references therein.
↓↑, Specific CEACAMs that are up- or downregulated in malignant versus healthy tissue specimens.
+, Greater than 50% of individual tumor samples were CEACAM positive.
(+), 10% to 50% CEACAM-positive tumor samples.

5
Biological Functions of CEACAMs

5.1
CEACAMs Are Cellular Adhesion Molecules

CEACAMs in human, mouse, and rat all act as homophilic cell adhesion molecules in vitro (Ocklind and Öbrink 1982; Rojas et al. 1990; McCuaig et al. 1992). Interestingly, despite its high homology to other CEACAMs, murine CEACAM2 does not function as a cell adhesion molecule (Robitaille et al. 1999).

The biological functions of the CEACAMs are largely determined by their ability to act as cellular and intercellular adhesion molecules. Adhesion of CEACAMs to each other or engagement with extracellular ligands induces specific signal transduction through CEACAMs, more specifically through CEACAM1-L and CEACAM3-L. Except for CEACAM3, CEACAM4, and CEACAM7, CEACAMs of the CEA-subgroup can interact with each other by homophilic or heterophilic binding. CEACAM1, CEA, and CEACAM6 exhibit homophilic and heterophilic adhesion among each other, whereas CEACAM8 seems to bind only to CEACAM6 (Oikawa et al. 1991). Binding of CEACAM1 to itself, CEA, or CEACAM6 was demonstrated by the use of recombinant CEACAM1 as well as in heterologous transfection. The adhesive properties of CEACAM1 are regulated by dimerization, the phosphorylation status of its cytoplasmic tail, its binding to calmodulin, and the activity of tissue transglutaminase (Öbrink 1997; Hunter et al. 1998). Recently, it was shown that adhesive properties of CEACAM1-4L can be modified after cleavage of its cytoplasmic tail by caspase-3 (Houde et al. 2003). Both the long-tail and short-tail isoform of CEACAM1 (CEACAM1-L and CEACAM1-S, respectively) undergo dimer formation in their cell-bound and soluble form, as shown using chemical crosslinkers (Hunter et al. 1996). Additionally, CEACAM1-L was identified as a substrate for tissue transglutaminase and it produces CEACAM1-L dimers by intracytoplasmic crosslinking (Hunter et al. 1998). CEACAMs preferentially form homodimers, i.e., CEACAM1-L binds to CEACAM1-L and CEACAM1-S binds to CEACAM1-S. Homodimerization of Ig superfamily members has been described for CD84, JAMs (junctional adhesion molecules), CD146, PECAM-1 (platelet endothelial cell adhesion molecule-1, CD31), ICAM-1 (intercellular adhesion molecule, CD54), N-CAM (neural cell adhesion molecule), and has also been established for cadherins (Rao et al. 1994; Shapiro et al. 1995; Nagar et al. 1996; Sun et al. 1996; Alais et al. 2001; Kostrewa et al. 2001; Martin et al. 2001). For cadherins and CEA, homodimer formation in *cis* is implicated in reinforcing adhesion of cadherins and CEA (Bates et al. 1992; Shapiro et al. 1995; Nagar et al. 1996).

In epithelial cells, a chemical equilibrium between dimers and monomers exists. This chemical equilibrium is influenced by the proliferative status

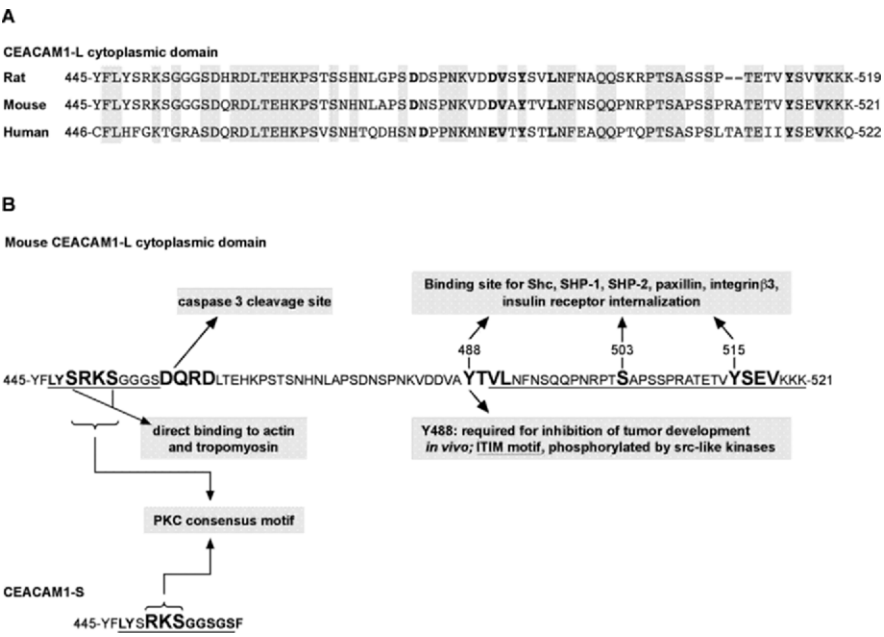

Fig. 5. **A** Comparison and amino acid sequence alignment of the highly homologous CEACAM1-L cytoplasmic domain in rat, mouse, and human. Conserved residues are *highlighted*. Conserved elements of signal transduction motifs are shown in *bold type*: the CEACAM1-L cytoplasmic domains contain conserved motifs containing tyrosine residues that are embedded into an imperfect ITAM (D/ExxxxxxD/ExxYxxLxxxxxxYxxL/I) or two perfect ITIMs (S/I/V/LxYxxL/V/I). Though the rat and mouse CEACAM1-L cytoplasmic domains are slightly shorter than the human CEACAM1-L cytoplasmic domain, Tyr513 in rat corresponds structurally and functionally to Tyr515 in mouse and Tyr516 in man. **B** Schematic summary of signal transduction and ligand binding properties of CEACAM1-S and CEACAM1-L cytoplasmic domains. Binding sites for calmodulin are *underlined*, binding sites for cytoskeletal components such as tropomyosin or actin, growth factor receptors or enzymes such as protein kinases or phosphatases and other intracellular adaptor proteins are *highlighted* appropriately. Residues known to be subject to phosphorylation and dephosphorylation are Tyr488, Ser503, and Tyr515. These residues are key mediators of CEACAM1-L signal transduction and adhesion

of the cell, the overall expression levels of the two isoforms, the ratio of CEACAM1-L and CEACAM1-S, and the levels of phosphorylation of the cytoplasmic tail on tyrosine and serine residues (Edlund et al. 1996; Öbrink 1997). CEACAM1-L and CEACAM1-S contain phosphorylation target sites for protein kinase C (PKC) that are located in proximity to calmodulin binding sites (Edlund et al. 1996). Calmodulin has two binding sites in CEACAM1-L and one in CEACAM1-S. The increase of intracellular calcium during cellular activation processes leads to the dissociation of CEACAM1 dimers in vitro (Öbrink 1997; Fig. 5).

In each case of *cis*- or *trans*-interaction between CEACAMs, their first Ig domain is essential (Cheung et al. 1993; Teixeira 1994; Watt et al. 2001). Homodimers interact through reciprocal bonds in their N-terminal domain (Wikström et al. 1996).

Examples for CEACAM function mediated by homophilic adhesion events include the establishment of tissue organization during embryonic development in the intestinal epithelium and in hepatocytes, in placental trophoblasts, during odontogenesis and myogenesis, during vascularization of the central nervous system, in neutrophil activation and extravasation during inflammatory responses, in regulation of T cell responses, in angiogenesis and regulation of cell proliferation (Öbrink 1997; Hammarström 1999; Zimmermann 2002). Examples of heterophilic interactions that involve CEACAMs are the adhesion to other CEACAM family members, adhesion to E-selectin, galectin-3, outer membrane proteins of *Neisseria meningitidis* and *Neisseria gonorrhoe*, *Haemophilus influenzae*, fimbriae of *Salmonella typhimurium* and *Escherichia coli*, and murine hepatitis virus, as described in detail below.

5.2 CEACAMs Display Versatile Signal Transduction Properties

5.2.1 Mechanisms of Signal Transduction by CEACAM1

The primary structure of the cytoplasmic domain displays a high conservation between humans and rodents. The most remarkable feature of the CEACAM1-L cytoplasmic domain lies in the two ITIM motifs (immunoreceptor tyrosine-based inhibition motif) that are fundamental in the regulation of signal transduction through CEACAM1-L. The CEACAM1-L cytoplasmic domain contains two tyrosine residues that have been shown to be phosphorylated. The first tyrosine, Tyr488, and the second tyrosine, Tyr515, are each located within an ITIM. Tyr515 in mouse corresponds to Tyr513 in rat and Tyr516 in human. ITIM motifs occur in a wide range of actual and potential coinhibitory receptors, in cytokine receptors or signaling kinases and intermediates. ITIMs are identified by the restricted consensus V/IxYxxL/V, but this sequence may be rather generally defined by the sequence V/I/L/SxYxxL/V/I (Sinclair 2000). When considering Tyr488 and Tyr515 together, these residues are located within an imperfect ITAM (immunoreceptor tyrosine-based activation motif; Fig. 5). In contrast to classic ITAMs, the spacing between the two tyrosine residues is not 10 amino acids, but rather 24–26 amino acids (Cambier 1995). The membrane proximal residue within the first ITIM, Tyr488, is phosphorylated by kinases of the src family, and by growth factor receptor tyrosine kinases such as the insulin receptor (IR) and the epidermal growth factor (EGF) receptor (Phillips et al.

1987). In epithelial cells, CEACAM1-L is phosphorylated by c-src, and in granulocytes by c-src, lyn, and hck (Skubitz et al. 1995). In hepatocytes, CEACAM1-L becomes tyrosine phosphorylated upon interaction with the IR tyrosine kinase (Margolis et al. 1990). In vitro, these tyrosine residues can be phosphorylated after treatment of cultured cells with the tyrosine phosphatase inhibitor pervanadate (Lin et al. 1995; Beauchemin et al. 1997). Regulated phosphorylation on Tyr488 of its cytoplasmic domain is of fundamental importance for signal transduction through CEACAM1-L. After phosphorylation on this residue, CEACAM1 interacts with components of the cytoskeleton, such as actin and paxillin, but also β_3 integrin (see below; Ebrahimnejad et al. 2000; Sadekova et al. 2000; Brümmer et al. 2001; Schumann et al. 2001).

Additionally, tyrosine phosphorylated CEACAM1-L becomes a substrate for the ubiquitously expressed phosphotyrosine phosphatases Src homology 2 domain-containing protein tyrosine phosphatase (SHP)-1 and SHP-2 (Beauchemin et al. 1997; Huber et al. 1999). Phosphorylation on both CEACAM1-L Tyr488 and Tyr515 is required for the recruitment of these SH2 domain-containing phosphatases. Binding of SHP-1 and SHP-2 has been described for coinhibitory receptors and other adhesion molecules that contain ITIM motifs in their cytoplasmic portion, such as a number of hematopoietic cell surface receptors: the Fcγ receptor IIB, CD22, and KIRs (killer inhibitory receptors) on natural killer cells (Long 1999; Sinclair 2000). Binding of SHP-2 to platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31) is also well characterized. Strikingly, the cytoplasmic domains of PECAM-1 and CEACAM1-L share a high homology within their C-terminal region (Huber et al. 1999). Both the PECAM-1 and CEACAM1-L cytoplasmic domains are transiently phosphorylated on two tyrosine residues during adhesion processes, or, as for PECAM-1, during platelet aggregation (Famiglietti et al. 1997). Abrogation of tyrosine phosphorylation on PECAM-1 can modulate its homophilic and heterophilic binding properties. A very similar model for regulated CEACAM1 dimerization and its subsequent association with intracytoplasmic adaptor proteins like serine and tyrosine kinases or phosphatases has been proposed by Öbrink's group (Öbrink et al. 2002 and references therein). Clustering of CEACAM1-L through monoclonal antibodies induces transient dephosphorylation (Budt et al. 2002).

In addition to tyrosine phosphorylation, CEACAM1-L becomes phosphorylated on Ser503. Basal phosphorylation on Ser503 was first described on CEACAM1-L in context with its activity as a bile salt transporter in rat bile canalicular cells that is associated with a co-purifying ecto-ATPase (Sippel et al. 1994). Furthermore, Ser503 phosphorylation is essential during complex formation between CEACAM1-L and the IR, and for its tumor cell growth suppressive effects (see below). Ser503 phosphorylation, however, appears to be regulated in part through tyrosine phosphorylation, indicating

that distinct signals of different signal transduction pathways can act through CEACAM1-L.

Although CEACAM1-L has been identified as the major active splice variant of CEACAM1 in signal transduction, CEACAM1-S also exerts interesting signal transduction properties, such as interaction with cytoskeletal components (see below), and phosphorylation on serine and threonine residues by protein kinase C isoenzymes (Edlund et al. 1998; cf. Fig. 5). However, its biological function is less well studied. In the mammary gland, CEACAM1-4S mediates apoptosis during mammary morphogenesis, and re-expression of CEACAM1-4S in breast cancer cell lines reverts their malignant phenotype to a normal phenotype when grown in 3D culture matrices (Kirshner et al. 2003a). Additionally, annexin II, a regulator of the secretory differentiation of mammary gland cells, has been identified as an intracellular ligand for CEACAM1-S (Kirshner et al. 2003b).

5.2.2 CEACAM1 Interacts with Components of the Cytoskeleton

In brush border cells of rat small intestine and in skeletal muscle cells, CEACAM1-L is associated with cortical actin, providing evidence for the implication of CEACAM1 in the organization and maintenance of tissue architecture (Hansson et al. 1989; Da Silva-Azevedo et al. 1999). It is assumed that this requires homophilic adhesion between CEACAM1 molecules on neighboring cells (Da Silva-Azevedo et al. 1999; Sadekova et al. 2000). Furthermore, CEACAM1-L also binds tropomyosin with its binding site in close proximity to the actin-binding site (Sadekova et al. 2000; Schumann et al. 2001). Using transfection studies in an adenocarcinoma cell line, it was discovered that the interaction with G-actin (globular actin) is dependent on tyrosine phosphorylation of the CEACAM1 long cytoplasmic tail. However, CEACAM1-S also contains actin-binding sites in its cytoplasmic portion. The individual sequences for actin binding in CEACAM1-S and CEACAM1-L are slightly different from each other. This might explain why tyrosine phosphorylation in CEACAM1-L can actually modulate its actin-binding properties (Schumann et al. 2001). The association with F-actin (fibrillar actin) can occur but is suspected to be indirect (Sadekova et al. 2000; Schumann et al. 2001). Importantly, CEACAM1-L localization in epithelial cells is restricted to their apical and lateral surface, i.e., cell–cell contacts (Sundberg and Öbrink 2002). This was also confirmed after microinjection of CEACAM1-L cDNA into fibroblasts and colon carcinoma cells (Sadekova et al. 2000). In contrast to CEACAM1-L, CEACAM1-S displays a rather diffuse localization in epithelial cells and fibroblasts after microinjection in a transient expression system (Sadekova et al. 2000). When stably transfected canine kidney epithelial cells (MDCK) are used, CEACAM1-S exhibits pronounced apical expression, but is not found in the lateral compartment. The explanation for this might be

the fact that the MDCK cells were allowed to form layers and were grown on filters that encourage cellular polarization (Sundberg and Öbrink 2002). In the microinjection experiments, CEACAM1-L and CEACAM1-S were co-expressed with constitutively active Rho–guanosine triphosphatases (GTPases) on untreated culture dishes (Sadekova et al. 2000). Rho GTPases control a variety of cellular processes such as organization of the cytoskeleton, gene transcription, and adhesion (Mackay and Hall 1998). It was revealed that Rho–GTPase activity was required to target CEACAM1-L to the cell periphery in epithelial cells, more specifically to sites of cell–cell contacts. Targeting of CEACAM1-L to cell–cell contacts is induced by activated cdc42 and Rac1, and requires the CEACAM1-L transmembrane domain (Fournes et al. 2003). This targeting could be abolished by use of chaotropic agents such as cytochalasin D that disrupts F-actin. No such effects on CEACAM1-S localization could be detected. Shively's group proposed a regulatory mechanism for actin polymerization through CEACAM1, assuming CEACAM1-S interacts with G-actin, serving as a putative G-actin polymerization site, and with CEACAM1-L as an anchoring platform for polymerized actin filaments (Schumann et al. 2001).

5.2.3 CEACAM1 Acts as a Tumor Suppressor

First indications pointing toward an involvement of CEACAM1 in suppression of tumor cell growth were obtained after observing differential regulation of CEACAM1 expression in hepatoma (Hixson et al. 1985). In hepatocarcinoma cells, loss of CEACAM1 expression was observed in malignant cells when compared to normal specimens (Tanaka et al. 1997). Later, it was discovered that CEACAM1 is also downregulated in other tumors of epithelial origin in human, mouse and rat, such as colon (Neumaier et al. 1993; Rosenberg et al. 1993; Nollau et al. 1997), prostate (Kleinerman et al. 1995a; Pu et al. 1999; Busch et al. 2002), breast (Riethdorf et al. 1997; Huang et al. 1998; Bamberger et al. 1998), endometrium (Bamberger et al. 1998), and bladder (Kleinerman et al. 1996), leading to the hypothesis that CEACAM1 behaves as a tumor suppressor. CEACAM1 downregulation is an early step during malignant transformation in human tumors, for example during the development of colon carcinomas, where its expression is markedly decreased in the microadenoma and adenoma stages (Ilantzis et al. 1997; Nollau et al. 1997). Intriguingly, a 1-bp deletion has been identified within a microsatellite region of the 3′ UTR of the human *CEACAM1* gene, which is suspected to be involved in tumor onset and progression by dramatically decreasing CEACAM1 expression (Ruggiero et al. 2003).

The antitumoral potential of CEACAM1 has been extensively investigated in cell culture and orthotopic xenograft models that provided further evidence for its suppressive effects on tumor cell growth in colon and prostate

cancer (Hsieh et al. 1995; Kleinerman et al. 1995b, 1996; Kunath et al. 1995; Luo et al. 1999; Estrera et al. 2001). Furthermore, it was revealed that the tumor suppressive effects exerted by CEACAM1-L in human, mouse, or rat are dependent on the presence of its long cytoplasmic tail and that physiological levels of CEACAM1 expression are required to sustain this function (Luo et al. 1997; Turbide et al. 1997; Izzi et al. 1999). The cytoplasmic tail of CEACAM1-L is necessary and sufficient to mediate growth inhibitory activity whereas CEACAM1-S does not display tumor cell growth inhibitory function (Turbide et al. 1997). These studies lead to the identification of amino acids that are crucial for the promotion of the reduction in tumor cell growth; as in other biological functions mediated by CEACAM1, phosphorylation on Tyr488, located within the ITIM, and on Ser503 play a key regulatory role in this context (Izzi et al. 1999; Estrera et al. 2001; Fournes et al. 2001).

Abrogation of tyrosine phosphorylation on Tyr488 or Ser503 by conversion of these amino acids to Phe or Ala, respectively, impairs the cell growth inhibitory effects. In contrast to Tyr488, tyrosine phosphorylation on Tyr515, as shown in mouse models, does not seem to influence the tumor suppressive potential of CEACAM1-L. The exact underlying molecular mechanisms for tumor suppression by CEACAM1 are still poorly understood. It is unclear at present whether dephosphorylation of the CEACAM1-L cytoplasmic domain by the tyrosine phosphatases SHP-1 and SHP-2 or a serine phosphatase is necessary in vivo to reduce tumoral cell proliferation.

Another aspect of tumor suppression by adhesion molecules is the phenomenon of contact inhibition leading to an arrest in cellular proliferation. In a cell culture model comparing a cell line derived from normal prostate epithelium and a bladder cancer cell line, homophilic cellular adhesion through CEACAM1 in *trans* and the expression of its isoform ratios was shown to be different in quiescent and proliferating cells (Singer et al. 2000). As mentioned above, the abilities of CEACAM1 to interact with components of the cytoskeleton and src-kinases or tyrosine phosphatases depend on the isoform ratio expressed by a particular cell type and engagement of these isoforms in dimer formation. The delicate equilibrium of CEACAM1-L monomers versus CEACAM1-L dimers regulates the balance between proliferative or anti-proliferative effects (Öbrink et al. 2002).

In general, few examples are known which reveal a causal context between the impact of signal transduction through adhesion molecules and malignant progression and dissemination of cells from a primary tumor. Well-studied examples in this context are E-cadherin and N-CAM (Birchmeier and Behrens 1994; Cavallaro et al. 2001; Hajra and Fearon 2002). Apart from the cadherin–catenin pathway, membrane association of the tyrosine phosphatases SHP-1 and SHP-2 can be observed in contact-inhibited cells. This association is required for E-cadherin-mediated growth arrest through $p27^{Kip}$, an effector of cyclin E-dependent kinase activity (Pallen and Tong 1991; St Croix

et al. 1998). Interestingly, CEACAM1 expression on quiescent, confluent bladder carcinoma cells correlates with the expression of $p27^{Kip}$ (Singer et al. 2000), whereas $p27^{Kip}$ is downregulated in proliferating cells. Additionally, CEACAM1 expression in human breast carcinomas is associated with the expression of tumor suppressors such as Rb (retinoblastoma protein) and $p27^{Kip}$ (Bamberger et al. 2002). Furthermore, adhesion molecules like cadherins and N-CAM respond to signaling by growth factor receptors, such as EGF and fibroblast growth factor (FGF), respectively. During tumor progression, their adhesive properties are modulated by the influence of these growth factors or, as it has been described for E-cadherin, their expression is downregulated upon EGF receptor activation (Hazan and Norton 1998; Cavallaro et al. 2001; Al Moustafa et al. 2002). CEACAM1 is also a substrate for growth factor receptors or responds to growth factor stimuli (Phillips et al. 1987; Ergün et al. 2000).

CEACAM1 is not downregulated in all carcinomas; its expression can also be upregulated in certain tumors, as mentioned above. Intriguingly, CEACAM1 expression also correlates with β_3 integrin expression in regulated and dysregulated invasive processes such as trophoblast invasion and the progression of malignant melanoma (Bamberger et al. 2000; Thies et al. 2002). The influence of putative interacting partners for CEACAM1 in *cis* and *trans* on the invasive behavior of primary tumors has not been investigated in detail so far. β_3 Integrin is the only *cis*-interacting adhesion molecule for CEACAM1-L identified to date (Brümmer et al. 2001). Moreover, the expression pattern of the CEACAM1 isoforms CEACAM1-S and CEACAM1-L in the majority of malignant human tumors has not been defined yet. In invasive human lung adenocarcinomas, for example, CEACAM1-S expression is markedly upregulated when compared to normal tissue specimen that predominantly express CEACAM1-L (Wang et al. 2000).

5.2.4 CEACAM1 Promotes Invasion

Contradictory to the observations mentioned above, CEACAM1 is overexpressed in human invasive melanomas, primary lung tumors (squamous cell carcinoma, adenocarcinoma and small cell carcinoma), and stomach tumors (Kim et al. 1992; Ohwada et al. 1994; Kinugasa et al. 1998; Wang et al. 2000; Laack et al. 2002; Thies et al. 2002). In human malignant melanoma, CEACAM1 expression is strongest at the invasive front of the primary tumor, and is preserved in distant metastatic lesions (Thies et al. 2002). Furthermore, CEACAM1 expression correlates with the development of metastatic disease and is an independent prognostic parameter. A similar correlation between CEACAM1 expression and poor prognosis is found in adenocarcinoma of the lung (Laack et al. 2002). However, in metastatic lesions of lung tumors, *CEACAM1* mRNA expression appears to be decreased when com-

pared to its expression levels in primary tumors (Ohwada et al. 1994). Moreover, CEACAM1 associates in *cis* with integrin $\alpha_v\beta_3$ at the invasive front of malignant human melanoma and at the apical surface of glandular cells of pregnancy endometrium (Brümmer et al. 2001). The concentrated colocalization at the tumor–stroma interface of invading melanoma and in the transitional region from proliferative to invasive extravillous trophoblast of the maternal–fetal interface indicates a role for CEACAM1/integrin β_3 complexes in cellular invasion (Brümmer et al. 2001).

5.2.5 CEACAM1 Is a Substrate for the Insulin Receptor and Regulates Insulin Clearance

Cellular sensitivity towards insulin is regulated by insulin-induced internalization and recycling of its receptor. Upon internalization of the receptor–ligand complex, the receptor is recycled, whereas insulin is targeted for degradation by passage through endosomal and lysosomal compartments. CEACAM1-L has been identified as an endogenous substrate for the tyrosine kinase activity of the insulin and EGF receptors in rat hepatoma cells and rat liver cell membranes (Rees-Jones and Taylor 1985; Phillips et al. 1987). In contrast to other substrates of the insulin receptor (IR), CEACAM1-L does not directly bind to insulin-like growth factor (IGF)-1 (Najjar et al. 1997).

The stimulation of insulin-induced endocytosis of the IR by CEACAM1-L requires a coordinated sequence of phosphorylation events in the CEACAM1-L and the IR cytoplasmic tails. Upon activation of the IR by ligand binding, it is autophosphorylated on diverse tyrosine residues and CEACAM1-L is recruited into the IR endocytosis complex (Choice et al. 1998; Najjar et al. 1998). This process is dependent on basal phosphorylation of CEACAM1-L on Ser503 by an unidentified cAMP-dependent kinase (Najjar et al. 1995). Two functionally distinct domains in the IR intracellular portion promote the phosphorylation of CEACAM1-L and its physical interaction with the receptor complex. Mutational analysis revealed that Tyr1316 in the C-terminal region of the β-chain of the IR is essential to promote phosphorylation of CEACAM1-L on Tyr488 (Najjar et al. 1997). Phosphorylation of CEACAM1-L on this tyrosine mediates the interaction with another, yet unidentified intracellular adapter that facilitates binding of the receptor-endocytosis complex to adaptor protein 2 adaptin proteins in clathrin vesicles and thus targets insulin for degradation (Najjar 2002; Fig. 6).

To initiate internalization, however, CEACAM1-L phosphorylation is required but not sufficient. Najjar et al. showed that phosphorylation on a conserved tyrosine residue in the IR juxtamembrane domain is fundamental in this process (Najjar et al. 1998). Transfection studies in heterologous systems confirmed that transfection with CEACAM1-L is sufficient to induce IR internalization (Soni et al. 2000).

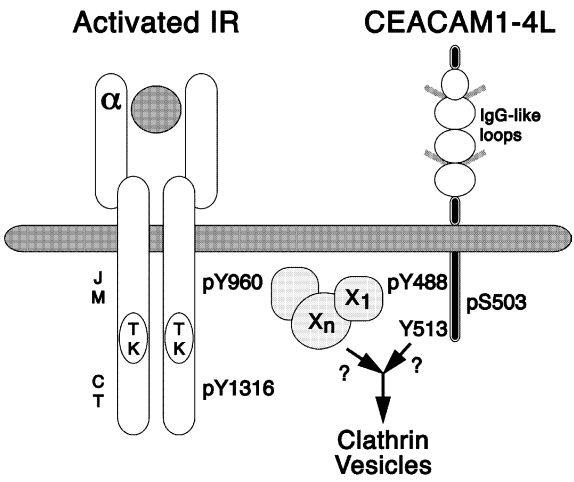

Fig. 6. Proposed model of receptor-mediated insulin endocytosis in the rat. Activation of the tyrosine kinase (*TK*) of the insulin receptor (*IR*) by insulin binding phosphorylates the receptor at many sites, including Tyr960 (*Y960*) in the juxtamembrane domain (*JM*) and Y1316 in the cytoplasmic portion (*CT*) of the β-subunit of the IR. Phosphorylation of Y1316 regulates phosphorylation of CEACAM1-4L on Y488. This causes CEACAM1-4L binding to an intracellular molecule (X_1) which mediates its indirect interaction with phosphorylated Y960 in the IR. Molecule X_i might function either alone or as a part of a complex of proteins (X_n). CEACAM1-4L, through Y513 and/or the mediator proteins, might then target the insulin endocytosis complex to AP2 adaptin proteins in clathrin vesicles to target insulin for degradation. Reproduced from Najjar 2002 with permission from Elsevier Science

CEACAM1-L downregulates the mitogenic effects of insulin by regulating insulin clearance but also through the interaction with Shc, an adaptor molecule implicated in inhibition of cellular proliferation. For the interaction with Shc, however, phosphorylation of CEACAM1-L by the IR is crucial (Poy et al. 2002a).

The in vivo regulatory effects of CEACAM1-L on insulin clearance were shown in a transgenic mouse model consisting of L-SACC1-transgenic mice that express a mutant form of rat CEACAM1-L (Ser503Ala) which cannot be phosphorylated on Ser503. The liver-targeted expression of this mutant under the ApoAI lipoprotein promoter caused secondary insulin resistance resulting from impaired insulin clearance by IR internalization in the mice (Poy et al. 2002b). Moreover, the CEACAM1-L Ser503Ala mutant exerts dominant-negative effects on the naturally expressed wildtype form of CEACAM1-L, emphasizing the immediate impact of signal transduction through CEACAM1-L in IR endocytosis and insulin clearance. The transgenic animals develop visceral adiposity with elevated plasma free-fatty acids

and plasma and hepatic triglyceride levels, resembling the phenotype of type II diabetes.

5.3 CEACAMs Are Modulators of the Innate and Adaptive Immune Response

5.3.1 CEACAM1 Is a Positive Regulator of Neutrophil Effector Function

CEA family members expressed on neutrophilic granulocytes include CEACAM1-L (CD66a), CEACAM3 (CD66d), CEACAM6 (CD66c), and CEACAM8 (CD66b). CEACAM1-L was initially described as a phosphoprotein with a relative molecular weight of 160 kDa on human neutrophilic granulocytes with crossreactive antibodies recognizing various members of the CD66 cluster of differentiation antigens (Skubitz et al. 1992). Its identity as CEACAM1 or CD66a could be revealed after a more detailed study by Wagener's group using monoclonal antibodies directed against CEA family members on human neutrophils (Drzeniek et al. 1991; Stoffel et al. 1993). CEACAM1 is an activation antigen that is upregulated rapidly from intracellular storage components after stimulation (Watt et al. 1991; Kuroki et al. 1995). In neutrophil development, CEACAM1 is expressed from the myelocyte stage and maintained throughout neutrophil differentiation (Elghetany 2002). It is detected in low levels on resting cells, but its expression is rapidly upregulated after stimulation with chemotactic peptides such as formyl-methionyl-leucyl-phenylalanine (fMLP), or calcium ionophores and phorbol esters (Skubitz et al. 1992). Upregulation of CEACAM1 in inflammatory disease has also been reported (Honig et al. 1999).

Upon neutrophil activation, CEACAM1-L becomes tyrosine phosphorylated and binds to protein kinases such as hck, lyn, and src (Brümmer et al. 1995; Skubitz et al. 1995). After phosphorylation, enhanced association of these kinases with the cytoskeletal components of neutrophils can be observed. Ligation of CEACAM1-L with monoclonal antibodies or $F(ab')_2$ fragments leads to upregulation of β_2 integrin (CD11b/CD18) expression and activation, production of cytotoxic oxygen species, downregulation of L-selectin, and increase of neutrophil adhesion to endothelial cells and fibrinogen and fibronectin (Stocks et al. 1995; Skubitz et al. 1996; Stocks et al. 1996; Klein 1996; Ruchaud-Sparagano et al. 1997; Nair and Zingde 2001). Homotypic adhesion of neutrophils to HUVECs (human umbilical vein endothelial cells) and subsequent activation of integrins can be enhanced by synthetic peptides containing sequences of the N-terminal domain of CEACAM1-L (Skubitz et al. 2001). Since CEACAM1-L is also expressed on activated HUVECs, homotypic adhesion via CEACAM1-L could further enhance adhesion to endothelia and trigger signal transduction pathways that may modulate integrin-binding activity.

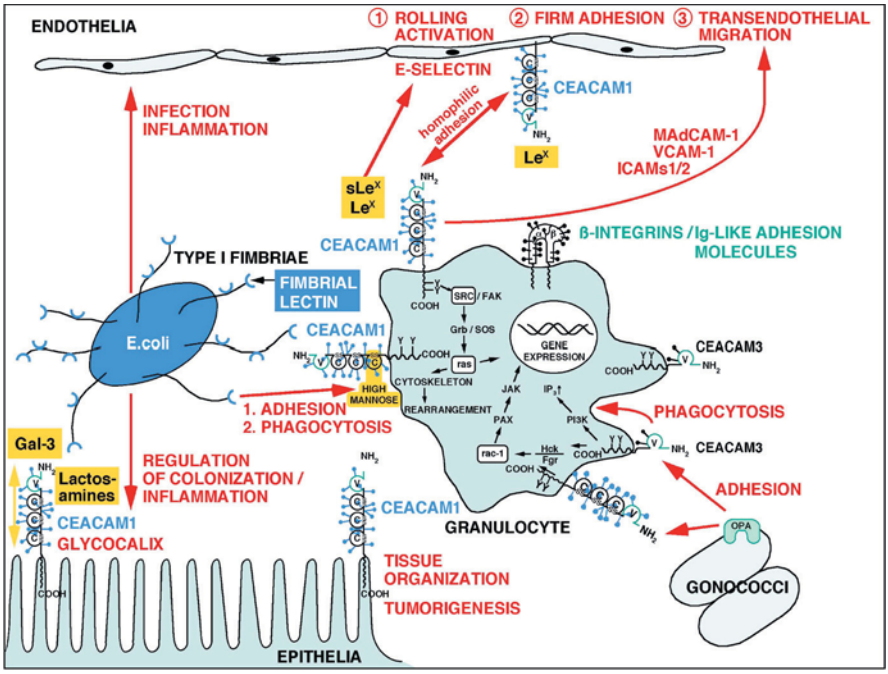

Fig. 7. Involvement of CEACAMs in the regulation of tissue colonization and infection by microbes through protein–carbohydrate and protein–protein interactions, as shown representatively for type 1-fimbriated *E. coli* and Opa^{+} gonococci. Carbohydrate moieties displayed by CEACAMs might be key players in fine-tuning interactions between individual CEACAMs or CEACAMs and their physiological ligands. CEACAM1 and CEACAM3 signal transduction capacities upon microbe binding are exemplified for neutrophilic granulocytes; please note that signal transduction conveyed by CEACAMs through specific signal transduction adaptor molecules such as src family kinases or GTPases of the Rho family also accounts for epithelia and endothelia. *Gal-3*, galectin-3; *Le^{x}*, Lewis^{x}; *sLe^{x}*, sialyl Lewis^{x}; *OPA*, opacity protein

On human neutrophils, CEACAM1 is the main carrier of complex oligosaccharides, such as type I and type II lactosaminoglycan chains. Since it is the main carrier of Lewis^{x} and sialyl-Lewis^{x}-epitopes on human granulocytes, CEACAM1 has been suggested as a lectin receptor for cellular lectins, such as vascular selectins. Lectin–glycoconjugate interactions initiate tethering of lymphocytes and granulocytes to the vascular surface, allowing subsequent firm adhesion and facilitating extravasation into inflamed tissues at sites of bacterial assault (McEver 2002). Furthermore, CEACAM1 is a receptor for the soluble mammalian lectin galectin-3 that mediates protein binding to extracellular matrix components (Stocks et al. 1990; Stocks and Kerr 1993; Ochieng et al. 1998; Feuk-Lagerstedt et al. 1999; Fig. 7).

5.3.2
CEACAM1 Regulates T and B Lymphocyte Function

CEACAM1-L is expressed on human and murine T and B lymphocytes, dendritic cells, and natural killer (NK) cells (Khan et al. 1993; Möller et al. 1996; Kammerer et al. 2001; Boulton and Gray-Owen 2002). CEACAM1-L is the only member of the CEA family with a cytoplasmic domain that has been characterized on B and T lymphocytes (Singer et al. 2002). On NK cells and T lymphocytes, CEACAM1 expression is inducible after stimulation, whereas B lymphocytes express CEACAM1 constitutively. In primary mouse B lymphocytes, CEACAM1 displays a distinct isoform expression pattern in resting and activated B cells: in concert with surface IgM-crosslinking, CEACAM-L triggers B cell proliferation, Ig secretion and β_2 integrin-mediated homotypic adhesion (Greicius et al. 2003). In resting T cells, CEACAM1 is present in intracellular stores and only in very low abundance on the cell surface; but as an activation antigen, it is rapidly upregulated after ligation of the TCR/CD3 complex or stimulation with interleukin (IL)-2 (Möller et al. 1996; Kammerer et al. 1998). Dendritic cells express both CEACAM1-S and CEACAM1-L. Two splice variants of CEACAM1 are expressed on human T lymphocytes—CEACAM1-3L and CEACAM1-4L—that are glycosylated in a lymphocyte-specific manner (Kammerer et al. 1998). The mechanism for CEACAM1 release from intracellular compartments might be analogous to that revealed for CTLA4, a coinhibitory receptor that is also retained in the cytoplasm but becomes rapidly translocated to the cell surface upon T cell activation and tyrosine phosphorylation of its cytoplasmic domain. Retention of CTLA4 in the cytoplasm is regulated by complex formation with adaptor protein 1, also called AP47. AP47 regulates the sorting of transmembrane proteins between the trans-Golgi network and the endosomal compartment. Tyrosine phosphorylation is not compatible with AP47-complex formation. Interestingly, Blumberg et al. showed that CEACAM1-L in murine T cells can bind SHP-1 and AP47 in its phosphorylated and dephosphorylated state, respectively (Nakajima et al. 2002). Once exposed on the cell surface, CEACAM1-L ligation by monoclonal antibodies or F(ab) fragments stimulates IL secretion by intestinal T lymphocytes (IL-7 and IL-15) and release of specific chemokines and interleukins by dendritic cells, such as macrophage inflammatory protein 1α, and ~2, monocyte chemoattractant protein 1, and IL-6 and IL-12 (Donda et al. 2000; Kammerer et al. 2001). In addition, CEACAM1-L-induced signaling increases the surface expression of costimulatory molecules (CD40, CD54, CD80, CD86), indicating a regulatory role for CEACAM1 in dendritic cell maturation and activation (Kammerer et al. 2001). The release of these cytokines leads to priming of naïve MHCII-restricted $CD4^+$ T cells with a T helper 1 effector phenotype, favoring a humoral immune response (Kammerer et al. 2001). In intestinal T lymphocytes,

cellular activation through CEACAM1-L induces an increase of nuclear factor (NF)-κB- and AP1-mediated transcription (Donda et al. 2000).

Contradictory to these positive regulatory effects on T cell function, CEACAM1-L can also act as a negative regulator of T cell responses. The above-mentioned reports describe regulatory influences of CEACAM1-L on the immune response as effects of either ligation of CEACAM1-L by specific antibodies or as secondary effects after stimulation with interleukins and chemokines or activation of the TCR/CD3 complex. However, a direct function for CEACAM1-L as a coinhibitory receptor on T lymphocytes was shown by Gray-Owen's group: The authors revealed that neisserial binding or binding of the neisserial Opa52 protein to its receptor CEACAM1-4L (see below) can arrest the activation and proliferation of $CD4^+$ T lymphocytes while the expression of the T cell activation marker CD69 is suppressed. Ligation of CEACAM1-L alone was shown to be sufficient for the suppression of $CD4^+$ T cell activation, an effect that could be enhanced after co-ligation of the ITAM-containing CD3ϵ chain. Furthermore, engagement of *Neisseria* with their receptor induces binding of the phosphatases SHP-1 and SHP-2 to the ITIM in the CEACAM1-L cytoplasmic domain. The reduction of T cell proliferation is a result of specific arrest in cell division and was not mediated through bacterial cytotoxicity (Bradbury 2002; Boulton and Gray-Owen 2002; Normark et al. 2002). Similarly, CEACAM1-L ligation with monoclonal antibodies can mediate negative regulation in T lymphocytes that results in inhibition of the delayed type hypersensitivity (DTH) reaction in early phases of T cell priming (Nakajima et al. 2002). Furthermore, CEACAM1-L ligation leads to downregulation of the cytolytic function of small intestinal intraepithelial lymphocytes ($CD8ab^+$/$TCRab^+$/CD28) and inhibits their CD3-directed and lymphokine–activated killer activity (Morales et al. 1999)

5.3.3 CEACAM1 Is a Novel Co-inhibitory Receptor on Human Natural Killer Cells

NK cells, such as neutrophils, belong to the innate immune defense and act by killing virus-infected or tumor cells in a MHC-I-dependent fashion. Their cytotoxicity is regulated by inhibitory, class I MHC-recognizing receptors that contain ITIM motifs in their cytosolic tail such as C-type lectins (CD94/NKG2A), killer Ig-related receptors, and the leukocyte Ig-like receptor. Among NK-specific receptors (NCRs), CD16 is implicated in mediating direct natural NK cell cytotoxicity (Long 1999; Mandelboim et al. 1999).

Natural killer cells that are freshly isolated express very low amounts of CEACAM1-L. However, CEACAM1-L-expression can be induced upon stimulation (Möller et al. 1996). CEACAM1-L is expressed on a subpopulation of activated human NK cells that are negative for CD16 ($CD16^-$) but positive for CD56 ($CD56^+$). Homotypic adhesion of CEACAM1-L on $CD16^-$/$CD56^+$ NK cells leads to inhibition of NK cell-mediated cytotoxicity in a novel

MHC-I-independent mechanism on a MHC-I-deficient melanoma cell line (Markel et al. 2002a). The degree of inhibition of CEACAM1-L on NK cytotoxicity correlates with the amount of CEACAM1-L expressed on the cell surface of target and effector cells. This leads to suppression of the adaptive and innate immune response to the tumor and provides a mechanism for tumoral evasion from immune surveillance. Recently, it was discovered that patients suffering from transporter associated with antigen processing 2 (TAP2) deficiency express unusually high levels of KIRs and CEACAM1, and that CEACAM1 exerts killer inhibitory function on these NK cells. This compensatory mechanism for class I MHC-dependent abrogation of killer activity leads to the inhibition of killing of tumor and autologous cells (Markel et al. 2003). Another situation that requires tight regulation of NK cell activity and suppression of their killer function is the invasion of the uterine endometrium by the extravillous trophoblast (Moffett-King 2002). $CD16^-/CD56^+$ NK cells constitute about 40% of decidual cells that upregulate CEACAM1-L expression upon stimulation with IL-2. Mandelboim's group showed that CEACAM1-L has a strong impact on the regulation of the local decidual immune response: CEACAM1-L-mediated homotypic interactions inhibit lysis, proliferation, and cytokine secretion of activated decidual NK, T, and NKT cells ($CD3^+/CD56^+$; Markel et al. 2002b). This allows maternal allorecognition of the fetus by uterine natural killer cells and invasion of the fetal extravillous trophoblast without challenging a cytotoxic immune reaction.

5.4 CEACAMs Are Receptors for Microbes and Viruses

5.4.1 CEACAMs Are Receptors for *Salmonellae* and *Escherichia coli*

CEACAM1, CEA, and CEACAM6 display high-mannose residues that are targeted by mannose-specific microbial lectins such as type 1 fimbriae of *E. coli* and *Salmonella typhimurium*. Type 1 fimbriae contain a lectin that specifically binds to terminally reducing α-mannosyl moieties (Manα1–3Man) present on glycans on cell surface receptors (Eshdat 1978).

Type 1 fimbriae bind to CEACAM1, CEA, and CEACAM6 on human intestinal epithelia and CEACAM1 and CEACAM6 on human neutrophilic granulocytes in vitro and in vivo in a mannose-dependent manner (Leusch et al. 1990, 1991a,b; Sauter et al. 1991; A.K. Horst, C. Wagener, unpublished data). Interestingly, the high-mannose glycan epitope for fimbrial binding to CEACAM1 and CEACAM6 seem to reside in different domains of these molecules. By mutational and biochemical analysis, Wagener's group demonstrated that CEACAM6 contains high-mannose moieties within its N-terminal domain. In contrast to this, high-mannose residues on CEACAM1 from human granulocytes are located within its membrane-proximal A2 domain

(Mahrenholz et al. 1993). On human granulocytes, CEACAM1 is the only member of the CEA family that contains an A2 domain, and mannose moieties within its N-terminal variable-like domain have not been demonstrated so far.

Activation of granulocytes is an important mechanism of the first-line defense: Binding of fimbriated bacteria triggers degranulation, production of cytotoxic oxygen species, activation of protein kinase C, and phospholipids turnover and bacterial uptake by lectinophagocytosis (Bar-Shavit et al. 1977; Sharon 1987; Gbarah et al. 1989). Furthermore, binding of fimbriated bacteria to intestinal epithelia is an essential function of CEACAMs in the regulation of the colonization of the commensal flora (Hammarström and Baranov 2001; Fig. 7).

5.4.2 CEACAMs Are Receptors for Neisseria

Initial attachment of gonococci to mucosal epithelia and neutrophils is mediated by bacterial pili (McGee et al. 1983). A firm secondary adhesion with subsequent ingestion and penetration into phagocytes or subepithelial layers, however, requires the expression of specific outer membrane proteins, the colony-associated Opa (opacity) proteins. Opa proteins trigger efficient opsonin-independent uptake of gonococci into phagocytic and non-phagocytic cells. Expression of gonococcal Opa proteins is phase variable (Stern et al. 1986; Meyer and van Putten 1989). Differential tropism of gonococci is determined by the expression of specific Opa variants (Kupsch et al. 1993). So far, two distinct classes of Opa proteins have been identified based on differential binding activity to their cellular receptors. The first class (Opa50-variants) binds to heparin sulfate proteoglycan-containing syndecan receptors (Chen et al. 1995), vitronectin, and fibronectin (Dehio et al. 1998). The second class of specific Opa-determinants (Opa52) targets CEACAMs. Heterologous transfection studies with *CEACAM* cDNAs revealed that meningococcal virulence-associated Opa-proteins, which are expressed by more than 95% of clinical and mucosal isolates of meningococci and gonococci, bind the N-terminal domain of CEACAM1-L. Additionally, meningococcal strains expressing capsule and sialylated lipopolysaccharide are also capable of binding to CEACAM1-L (Virji 1996). Besides CEACAM1-L, CEACAM3-L, CEA, and CEACAM6 are targeted by Opa proteins of *Neisseria*. However, different Opa variants display overlapping binding specificities for CEACAMs—some bind to CEACAM1, CEACAM3, CEA, and CEACAM6, whereas the binding of others is restricted to both CEACAM1 and CEA or CEA alone (Chen et al. 1997; Gray-Owen et al. 1997a,b; Bos et al. 1998). In contrast to these findings, Virji et al. demonstrated that CEACAM6 was not bound by certain Opa variants of different neisserial strains. The binding epitope within the N-terminal domain that is targeted by *Neisseria* is con-

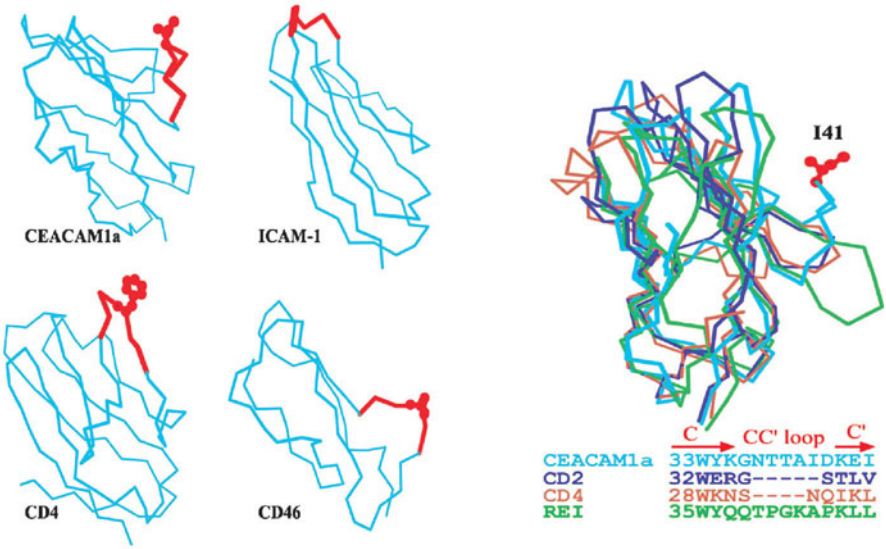

Fig. 8. Structural comparison of the immunoglobulin fold of the N-terminal domains of different members of the Ig superfamily which serve as receptors for various pathogens: soluble murine *CEACAM1a*, the receptor for murine coronavirus MHV; *ICAM-1*, the receptor for the major group of rhinoviruses; *CD4*, the primary receptor for HIV (human immunodeficiency virus); and *CD46*, the receptor for measles virus. The key virus binding residues are highlighted in *red*. Superposition (*right*) of the D1 domain of soluble murine CEACAM1a[1,4] (*cyan*), CD2 (*blue*), CD4 (*brown*) and REI (*green*). The unique convoluted conformation of the CC′ loop in soluble murine CEACAM1a[1,4] is striking. The sequence alignment of the CC′ loop regions of these four molecules are shown (*boted from Tan et al. 2002 with permission)*

fined to β-strand C in the non-glycosylated GFC face (cf. Fig. 8). Further studies revealed that differential tropism is mediated by a heterologous triplet of amino acids within this region (Popp et al. 1999).

Binding of Opa52$^+$ gonococci to CEACAM1-L provokes the respiratory burst in phagocytes and triggers tyrosine phosphorylation involved in CEACAM-mediated activation of the src-kinases Hck and Fgr (Hauck et al. 2000) and rapid activation of acid sphingomyelinase (Hauck et al. 2000). Acid sphingomyelinase activity is not only crucial for the activation of src-like kinases, but also for the small GTPase Rac1 (Grassme et al. 1997) and bacterial ingestion (Hauck 1998). Rac1 activates the stress-activated protein kinase pathway through PAK1 (p21-activated protein kinase) and JNK (Jun-N-terminal kinase; Hauck 1998; Fig. 7). Activation of src-like kinases and Rac1 drive cytoskeletal rearrangements that promote bacterial phagocytosis. This pathway is distinct from the opsonin-dependent Fcγ receptor-mediated signaling, since it does not involve Syk activation and is specific for Opa52$^+$ gonococci. Furthermore, the level of phosphorylation of the phos-

photyrosine phosphatase SHP-1 and thus its activity is modulated by Opa-induced events. Increased tyrosine phosphorylation of SHP-1 by src-like kinases results in downregulation of its activity, thus reducing inhibitory pathways that might interfere with the bacterial uptake (Hauck 1998; Hauck et al. 1999). Neisserial binding to epithelia and endothelia triggers upregulation of CEACAM1 through inflammatory cytokines, such as tumor necrosis factor (TNF)-α, and through NF-κB-activated transcription (Muenzner et al. 2001, 2002). By this mechanism, *Neisseria* induce signal transduction pathways that allow efficient colonization of host tissue and encourage bacterial engulfment and transcellular passage.

The mechanism underlying CEACAM3-L-mediated uptake of *Neisseria* seems to employ different mechanisms when compared to CEACAM1-L: Bacterial uptake and intracellular survival was discovered to be associated with PI3 K (phosphatidylinsositiol-3-kinase) activity (Booth et al. 2003). In contrast to CEACAM1-L, CEACAM3-L induces IP_3 (phosphatidyl-inositol(3,4,5) trisphosphate) accumulation at sites of bacterial internalization. Furthermore, downstream products of the PI3 K pathway, such as phosphatidylinositol 3-phosphate, seem to be involved in the regulation of bacterial survival, due to their requirement for phagosomal maturation (Booth et al. 2003). CEACAM3 is expressed in granulocytes only and may support innate host defense. Though CEACAM3-L shares a high homology with CEACAM1-L, the CEACAM3-L cytoplasmic domain contains an ITAM instead of an ITIM that binds tyrosine kinases of the src-family and the calprotectin complex, and supports bacterial engulfment in a phosphotyrosine-dependent manner (Chen 2001; Streichert et al. 2001; McCaw et al. 2003).

5.4.3 CEACAMs Are Receptors for *Haemophilus Influenzae*

CEACAMs are also targeted by numerous strains of capsulate (typable) and acapsulate (non-typable) *Haemophilus influenzae*. Like gonococci, *Haemophilus* binds to CEACAMs through a variable outer membrane protein, the P5 protein, and interacts primarily with the N-terminal domain of CEACAMs. However, several strains seem to require more than the presence of the N-terminal domain for efficient binding of soluble CEACAMs, such as additional A or B domains (Virji et al. 2000). Non-adherent strains can reconstitute their ability to bind both to purified CEACAM1 and CEACAM1-transfected cells (CHO cells, Chinese hamster ovary cells) after transformation with the P5 protein. In contrast to the adhesin P5, *Haemophilus* expresses additional ligands that might target CEACAM1 on cell surfaces (Hill et al. 2001). Strikingly, site-directed mutagenesis with substitution of the surface-exposed amino acids revealed that the binding epitope for diverse *Haemophilus influenzae* strains lies within the same exposed region of the

CFG face that also harbors the sequence targeted by *Neisseria* (Bos et al. 1999; Virji et al. 1999; Fig. 8).

5.4.4 CEACAMs Are Receptors for Murine Hepatitis Virus

Murine hepatitis virus (MHV) strain A59 belongs to the enveloped positive-stranded RNA viruses in the coronaviridae family in the order nidovirales. Murine hepatitis viruses cause respiratory and enteric infections, hepatitis immune dysfunction, acute encephalitis, splenolysis, and chronic demyelinating disease (Compton et al. 1993). CEACAM1 was identified as a receptor for the murine hepatitis virus by Williams et al., and the cDNA was cloned by Holmes' group (Williams et al. 1990, 1991; Dveksler et al. 1991). In contrast to humans, mice express two allelic variants of the *Ceacam1*-gene, *Ceacam1a* and *Ceacam1b*, which each give rise to four distinct isoforms emerging from differential splicing. These isoforms consist of two or four extracellular domains and a long or short cytoplasmic tail (73 or 10 amino acids, respectively). All four murine CEACAM1a proteins and CEACAM2, consisting of the N-terminal domain and the A2 domain, are receptors for murine hepatitis virus strain A59 when recombinant proteins are expressed in a heterologous system (Baby Hamster Kidney cells; Nedellec et al. 1994). CEACAM1a is a high-affinity receptor for the 180-kDa viral spike glycoprotein of the murine hepatitis virus. Human HV seems to utilize a different receptor when compared to murine HV (Gagneten et al. 1996).

Most inbred laboratory mouse strains are susceptible to MHV infection and are homozygous for the CEACAM1a allele. However, mice homozygous for the CEACAM1b allelic variant display weaker MHV-A59 binding activity and are MHV resistant, such as SJL/J mice. To date, CEACAM1a proteins (including their splice variants) are the only receptors for MHV (Dveksler et al. 1993a). As shown in heterologous transfection studies, high levels of CEACAM2 expression also induce susceptibility towards MHV, although CEACAM2 displays a significantly lower affinity for the virus when compared to the CEACAM1a proteins (Nedellec et al. 1994). The differential expression of the CEACAM1-isoforms in different mouse tissues may explain the tissue tropism of different MHV strains. The target epitope for the viral spike protein resides within the N-terminal domain of CEACAM1, and glycosylation of the three potential N-linked glycosylation sites is not required for viral binding (Dveksler et al. 1995). Efficient binding, however, requires a CEACAM1 protein that contains more Ig domains than just the N-terminal domain (Dveksler et al. 1993b). In a partial mouse knockout system (p/p mice), targeted disruption of the murine *Ceacam1* gene leads to a marked reduction of susceptibility towards MHV (Blau et al. 2001). The p/p mice do indeed get infected by the MHVA59 virus, but interestingly, the liver pro-

duces only small foci compared to those observed in the wildtype mice, and these foci disappear 5 days post-infection.

In comparison to other members of the Ig superfamily, CEACAMs exhibit unique structural features which determine their distinctive homophilic and heterophilic adhesion properties (Fig. 8). This was demonstrated by Holmes' group in the analysis of the crystal structure of soluble murine CEACAM1a[1,4]: Superposition of the CEACAM1 N-terminal domain on the variable-like domain of other representative members of the IgSF, CD2, CD4, and the REI protein, reveals a protruding loop interconnecting the β-strands C and C′ of CEACAM1-L that folds back onto the CFG face of the ABED β-sheet. The corresponding region in the REI protein, a typical variable domain of an antibody, however, depicts a hairpin-like structure (Tan et al. 2002; Fig. 8).

Comparison of the CEACAM1 N-terminal domain to the N-terminal domains of other virus receptors, like ICAM-I (intercellular cell adhesion molecule-I; CD54), a rhinovirus receptor, CD4, the primary receptor for HIV (human immunodeficiency virus), and CD46, the receptor for measles virus, reveals common structural features such as exposed hydrophobic residues (highlighted in red, left side of Fig. 8) that are crucial for viral binding and determine viral tropism (Wang and Springer 1998). In the CEACAM1 CC′ loop, an Ile (Ile41) becomes exposed, which is a highly conserved residue in human and rodent CEACAMs. This Ile lies within a peptide sequence that has been defined by mutational analysis as the target for MHV binding. Transferring the structural data obtained from murine CEACAM1 to human CEACAM1, it is possible to assume that the N-terminal domain of human CEACAM1 and other CEACAMs exhibit very similar structural properties, and that the CC′ loop also acquires a convoluted conformation (Tan et al. 2002). The importance of this conformational epitope also becomes evident in the context of neisserial interaction with CEACAMs, as well. The binding area on CEACAM1 and CEA targeted by *Neisseria* is constituted by amino acid residues on strand C, the CC′ loop and strand F. Conserved amino acids within this region are responsible for stabilizing the convoluted conformation of the CC′ loop (Fig. 8). Mutational analysis targeting these amino acids leads to abrogation of neisserial binding (Virji et al. 1999). In a different approach studying neutrophil adhesion to HUVECs, synthetic peptides containing amino acid sequences within the CC′ loop and turns between the β-sheets were found to trigger neutrophil adhesion through β_2 integrin activation to HUVECs (Skubitz et al. 2000; Skubitz et al. 2001). These data emphasize that homophilic adhesion processes mediated by CEACAM1 or binding to other or yet unidentified ligands in *trans* mediate CEACAM-functions in vivo.

Moreover, homophilic interactions between CEACAMs is mediated by interactions of the CC′ loop and the FG loops in the N-terminal domain. Interestingly, the ABED sheet is much more conserved in CEACAMs than the

CFG face, though the N-terminal domains of CEACAMs exhibit 70%–90% amino acid sequence identity. This might explain their selective binding properties and allows a certain flexibility for the interaction with different family members.

5.5 CEACAM1 Modulates Angiogenesis

5.5.1 CEACAM1 Is an Angiogenic Growth Factor

Angiogenesis, the sprouting outgrowth of blood vessels from pre-existing ones, is initiated by the angiogenic switch, a cascade triggered by a tip in the balance of pro-angiogenic and anti-angiogenic factors (Hanahan and Folkman 1996). Major regulatory stages in angiogenesis involve (1) the activation of endothelial cells by soluble growth factors that bind to their cognate receptor tyrosine kinases (Folkman 1996), (2) the initiation of endothelial cell migration and cellular interaction with extracellular matrix components, and (3) cellular proliferation and differentiation into endothelial tubes. Major growth factor families involved in angiogenesis are the vascular endothelial growth factor (VEGF)-receptor family, the tie-receptor family binding to angiopoietins, and the ephrins. Angiogenesis is initiated by signaling through one or several of these receptors either alone or in concert with adhesion molecules that are activated upon growth factor receptor signaling (Byzova et al. 2000; Carlson et al. 2001). In later stages of angiogenesis, however, adhesion molecules, i.e., immunoglobulins and integrins, are the major instruments orchestrating angiogenesis-related events such as cellular migration and the establishment of a vascular network on the basis of cellular adhesion and interaction with the extracellular matrix (Carmeliet and Jain 2000; Conway et al. 2001).

CEACAM1 is the only member of the CEA family with a cytoplasmic tail expressed on endothelia. Apart from CEACAM1, CEA is also expressed on endothelia (Majuri et al. 1994). Recently, CEACAM1 has been identified as an angiogenic growth factor (Ergün et al. 2000). In addition to growth factor receptors and their ligands, vascular adhesion molecules such as platelet/endothelial cell adhesion molecule (PECAM)1 (CD31) and the integrin $\alpha_V\beta_3$ also contribute to angiogenesis by triggering endothelial cell migration and mediating cell–cell and cell–extracellular matrix interactions (Ferrero et al. 1995; Varner et al. 1995). In this context, it is noteworthy that CEACAM1-4L interacts with integrin $\alpha_V\beta_3$ in human endothelial and epithelial cells (Brümmer et al. 2001). The first indication that CEACAM1 is involved in angiogenesis emerged from the findings that it is expressed on microvessels of proliferating tissue, such as microvessels in the developing central nervous system of the rat (Sawa et al. 1994) and in wound healing edges in granuloma tissue.

Furthermore, blood vessels on the fetal-maternal interface, i.e., in the decidua and the placenta, display CEACAM1 expression (Daniels et al. 1996; Prall et al. 1996; Bamberger et al. 2001). In the adult, CEACAM1 can be detected exclusively in small blood vessels, and no expression is found on mature, large blood vessels. In contrast to the distinct expression pattern in the human vasculature, CEACAM1 is expressed on the majority of large and small blood vessels in the mouse (A. Horst, N. Beauchemin, and C. Wagener, unpublished data). In human tumors, CEACAM1 is present in vessels of renal cell carcinoma, carcinomas of the urinary bladder and prostate, and Leydig cell tumors (Ergün et al. 2000).

CEACAM1 is present on capillary-like structures formed by endothelial progenitor cells when transplanted together with tumor cells into severe combined immunodeficiency (SCID) mice (Gehling et al. 2000). Purified native and recombinant CEACAM1 stimulates proliferation, chemotaxis, and tube formation of human microvascular endothelial cells and induces angiogenesis in the chorioallantois membrane (CAM) of the chicken. The angiogenic effects initiated by CEACAM1 were found to be additive in combination with VEGF or basic (b)FGF. Furthermore, stimulation of human dermal microvascular endothelial cells with VEGF and bFGF leads to induction of CEACAM1 expression, suggesting the existence of a functional link in a common signaling pathway (Ergün et al. 2000). As shown for the IR and the EGF receptor, CEACAM1 does act as a substrate for receptor tyrosine kinases (Phillips et al. 1987). The molecular link between vascular growth factor receptor signaling pathways and CEACAM1, however, still needs to be elucidated.

The fact that CEACAM1 is a major carrier of sialyl Lewis^x and Lewis^x residues on granulocytes suggests that CEACAM1 could function as a ligand for E-selectin (Kerr and Stocks 1992; Stocks and Kerr 1993; Sanders and Kerr 1999). Under physiological conditions, sialyl Lewis^x and Lewis^x-carrying glycoproteins support intercellular adhesion of endothelial cells (see also Fig. 7).

In summary, CEACAM1 exerts distinct functions in different stages of angiogenesis: during early stages, in its soluble form, it acts a chemoattractant, and its expression is upregulated in response to angiogenic stimuli (Ergün et al. 2000). Cleavage of cell-bound growth factor receptors and adhesion molecules has been described in the context of the regulation of their bioavailability and ligand-binding activities (Hornig et al. 2000; Carmeliet et al. 2001). In later stages of angiogenesis, as the nascent vasculature is stabilized, adhesion molecules are important tools for the recruitment of accessory cells, such as pericytes and the establishment of cell–cell junctions. CEACAM1 shares certain functional features with CD31 and junctional cell adhesion molecules (JAMs) that have been reported to support the organization of cell–cell junctions by homophilic interactions. These molecules do not only regulate vessel permeability and endothelial cell–cell interactions but also participate in cel-

lular communication during neutrophil transmigration (Ferrero et al. 1995; Dejana et al. 2001). Additionally, CEACAM1 may facilitate the establishment of endothelial cell–cell contacts through binding of E-selectin and participate in the recruitment of accessory cells. By organizing cellular adhesion, CEACAM1 has been shown to promote tube formation in human endothelial cells and the lumen formation of epithelial cells in an extracellular matrix, providing evidence that it is indeed a key player in organizing luminal tissue architecture and regulating cellular differentiation (Huang et al. 1998; Kirshner et al. 2003a).

5.5.2 CEACAM1 Induces Secretion of Angiostatic Factors in CEACAM1-Transfected Prostate Carcinoma Cells

Unexpectedly, it was discovered that adenovirus-mediated CEACAM1-L expression in prostate cancer cells triggered the synthesis of a yet unidentified soluble angiostatic factor (Volpert et al. 2002). This study had originally been designed to further investigate the inhibitory effects of CEACAM1-L on prostate cancer cell growth. It was hypothesized that the tumor-suppressive effects of CEACAM1-L on prostate cancer cells is a result of the inhibition of tumor angiogenesis and that secretion of an angiostatic factor is specifically induced by re-expression of CEACAM1-L in these cells. Cell culture medium conditioned by wildtype adenovirus-CEACAM1-L-infected prostate carcinoma cells inhibited human endothelial cell migration and corneal neovascularization in vivo as well as an increase of endothelial cells. This effect could not be produced with media conditioned by a CEACAM1-L mutant (Ser503Ala) that is also incapable of conferring tumor-suppressive effects or endothelial cell apoptosis (Volpert et al. 2002).

In contrast to the above-mentioned model systems, purified or soluble CEACAM1-L per se has not been tested with regard to its angiogenic properties. Furthermore, it has not been mentioned whether CEACAM1 expression by endothelial cells co-cultured with the transfected prostate carcinoma cells was reduced or if secretion of soluble CEACAM1 by the endothelial cells was impaired. These two different approaches, however, provide important clues on how CEACAM1 can affect angiogenesis with respect to endothelial cell outgrowth and differentiation but also how changes in CEACAM1 expression during tumor progression in certain epithelia influence angiogenic events.

6 Perspectives

CEACAM1 is the most ancestral member of the CEA gene family. Expression of CEACAMs starts early during embryogenesis, and is maintained throughout life. CEACAMs are fundamental for establishing and maintaining tissue architecture. Although CEACAMs function as cellular adhesion molecules, they are not static, but rather involved in a great variety of dynamic processes, such as growth factor signaling, regulation of immune responses, and tumor cell growth suppression, as well as promotion of invasion. The signal transduction properties of CEACAM1 and its effects on cellular proliferation and differentiation are determined by its engagement in cellular adhesion and the expression level of different isoforms (Öbrink et al. 2002). As a signaling molecule, CEACAM1 displays dual functions that are exploited differently in different cellular contexts. On neutrophilic granulocytes, CEACAM1-L acts as a positive stimulator inducing activation and adhesion of neutrophils to endothelial cells through β_2 integrins, and it mediates opsonin-independent phagocytosis. An example for a negative regulatory effect through CEACAM1-L signal transduction can be observed after neisserial binding to CEACAM1-L on T lymphocytes and results in arrest of T cell proliferation (Boulton and Gray-Owen 2002). As reported by Mandelboim's group, CEACAM1-L expression on melanoma cells provides a mechanism for tumor cells to evade immune surveillance by downregulation of NK cell responses through homophilic interactions mediated by CEACAM1-L in *trans* (Markel et al. 2002a). It is worth noting that CEACAM1 is the only member of the CEA family with a long cytoplasmic domain that is capable of homophilic adhesion. Another important functional aspect of CEACAMs is that expression of membrane-bound forms with a GPI-anchor is predominantly—but not exclusively—found on epithelia, whereas CEACAMs that contain a long cytoplasmic domain and are capable of active signal transduction are also expressed on a variety of motile and circulating cells, such as lymphocytes, granulocytes, dendritic cells, or endothelial cells. Here, it is striking that especially CEACAM1-L expression is subject to upregulation after cellular activation by cytokines, chemokines, microbial proteins, or growth factors. However, it must not be neglected that other members of the CEA family, like CEA, CEACAM6, as well as CEACAM1, respond to inflammatory cytokines on intestinal epithelial cells (Takahashi 1993).

CEA is an ubiquitous clinical marker with prognostic relevance in the evaluation of various progressive malignant carcinomas. Its qualities as a diagnostic clinical marker, target for anticancer vaccines, and its therapeutic use has been reviewed elsewhere (Stanners 1998; Bunjes et al. 2001; Berinstein 2002; Pagel et al. 2002). Recently, CEACAM1 expression on invasive melanoma and adenocarcinoma of the lung was discovered as a novel clinical marker of high prognostic significance that is directly correlated

with patient survival (Laack et al. 2002; Thies et al. 2002). In a multivariate analysis in lung cancer and melanoma patients, CEACAM1 was identified as an independent marker of poor prognosis and risk of metastasis (Laack et al. 2002; Thies et al. 2002; Sienel et al. 2003). In addition, the strongest CEACAM1 expression in malignant melanoma is observed on the invading front of the tumor. Its co-expression with β_3 integrin provides evidence for direct involvement of CEACAM1 in the regulation of invasive processes (Brümmer et al. 2001). In contrast to CEACAM1 upregulation in certain tumors, CEACAM1 expression is markedly reduced or even lost during early stages of tumor progression, such as in colonic carcinomas (Ilantzis et al. 1997; Nollau et al. 1997). In this case, tumor dissemination is encouraged by dramatic changes in tissue architecture and intercellular adhesion. Moreover, CEACAMs express Lewis blood group antigens that are associated with metastatic spread of primary tumors (Sanders and Kerr 1999). Besides its upregulation on tumoral tissues in a membrane bound form, CEACAM1 serum levels are elevated in jaundice and chronic liver diseases (Lucka et al. 1998; Draberova et al. 2000; Kondo et al. 2001). The effects of soluble CEACAM splice variants on the regulation of homophilic and heterophilic adhesion or their potential to act as soluble growth factors still need to be defined. It has not been investigated, however, if CEACAMs of the CEA family other than CEA and CEACAM6 are released from the cell membrane of normal or tumor cells and whether putative soluble forms have any implication in the regulation of adhesion or malignant progression. Furthermore, specific mutations associated with clinically relevant phenotypes have not been described yet except for CEA: patients with primary tumors and exceptionally high serum levels of CEA display a mutated form of CEA that is associated with increased hepatic metastasis (Zimmer and Thomas 2001).

With the perspective of developing new gene therapies that revert the malignant phenotype of primary tumors, a preclinical trial for the treatment of androgen-independent prostate cancer is currently under investigation. In this approach, adenovirus-mediated gene transfer of *CEACAM1-L* cDNA into PC-3 prostate carcinoma cells in a nude mouse xenograft model led to a marked reduction of tumor cell growth (Kleinerman et al. 1995b). In addition, the combination of *CEACAM1-L* gene therapy and treatment with the angiogenic inhibitor TNP-470 in this model resulted in enhanced suppression of tumor cell growth in vivo compared to restoration of CEACAM1-L expression alone (Pu et al. 2002). This study provides a novel therapeutic approach that takes advantage of the tumor suppressive effects of a cellular adhesion molecule and anti-angiogenic agents. It provides evidence that CEACAM1-L is a novel putative therapeutic agent for the treatment of malignant tumors.

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References

Al Moustafa AE, Yen L, Benlimame N, Alaoui-Jamali MA (2002) Regulation of E-cadherin/catenin complex patterns by epidermal growth factor receptor modulation in human lung cancer cells. Lung Cancer 37:49–56

Alais S, Allioli N, Pujades C, Duband JL, Vainio O, Imhof BA, Dunon D (2001) HEMCAM/CD146 downregulates cell surface expression of beta1 integrins. J Cell Sci 114:1847–1859

Bamberger AM, Riethdorf L, Nollau P, Naumann M, Erdmann I, Götze J, Brümmer J, Schulte HM, Wagener C, Löning T (1998) Dysregulated expression of CD66a (BGP, C-CAM), an adhesion molecule of the CEA family, in endometrial cancer. Am J Pathol 152:1401–1406

Bamberger AM, Sudahl S, Löning T, Wagener C, Bamberger CM, Drakakis P, Coutifaris C, Makrigiannakis A (2000) The adhesion molecule CEACAM1 (CD66a, C-CAM, BGP) is specifically expressed by the extravillous intermediate trophoblast. Am J Pathol 156:1165–1170

Bamberger AM, Sudahl S, Wagener C, Löning T (2001) Expression pattern of the adhesion molecule CEACAM1 (C-CAM, CD66a, BGP) in gestational trophoblastic lesions. Int J Gynecol Pathol 20:160–165

Bamberger AM, Kappes H, Methner C, Rieck G, Brümmer J, Wagener C, Löning T, Milde-Langosch K (2002) Expression of the adhesion molecule CEACAM1 (CD66a, BGP, C-CAM) in breast cancer is associated with the expression of the tumor-suppressor genes Rb, Rb2, and p27. Virchows Arch 440:139–144

Bar-Shavit Z, Ofek N, Goldman R, Mirelman D, Sharon N (1977) Mannose residues of phagocytes as receptors for the attachment of *E. coli* and *Salmonella typhi*. Biochem Biophys Res Commun 78:455–460

Baranov V, Yeung MM-W, Hammarström S (1994) Expression of CEA and nonspecific cross-reacting 50 kD Antigen in human normal and cancerous colon mucosa: comparative ultrastructural study with monoclonal antibodies. Cancer Res 54:3305–3314

Barnett TR, Kretschmer A, Austen DA, Goebel SJ, Hart JT, Elting JJ, Kamarck ME (1989) Carcinoembryonic antigens: alternative splicing accounts for the multiple mRNAs that code for novel members of the CEA family. J Cell Biol 108:267–276

Barnett TR, Drake L, Pickle W 2nd (1993) Human biliary glycoprotein gene: characterization of a family of novel alternatively spliced RNAs and their expressed proteins. Mol Cell Biol 13:1273–1282

Bates PA, Luo J, Sternberg MJ (1992) A predicted three-dimensional structure for the carcinoembryonic antigen (CEA). FEBS Lett 301:207–214

Baum O, Troll S, Hixson DC (1996) The long and the short isoform of cell-CAM 105 show variant-specific modifications in adult rat organs. Biochem Biophys Res Commun 227:775–781

Beauchemin N, Benchimol S, Cournoyer D, Fuks A, Stanners CP (1987) Isolation and characterization of full-length functional cDNA clones for human carcinoembryonic antigen. Mol Cell Biol 7:3221–3230

Beauchemin N, Kunath T, Robitaille J, Chow B, Turbide C, Daniels E, Veillette A (1997) Association of biliary glycoprotein with protein tyrosine phosphatase SHP-1 in malignant colon epithelial cells. Oncogene 14:783–790

Beauchemin N, Draber P, Dveksler G, Gold P, Gray-Owen S, Grunert F, Hammarsröm S, Holmes KV, Karlsson A, kuroki M, Lin S-H, Lucka L, Najjar SM, Neumaier M, Öbrink B, Shively JE, Skubitz KM, Stanners CP, Thomas P, Thompson JA, Virji M, von Kleist S, Wagener C, Watts S, Zimmermann W (1999) Redefined nomenclature for members of the carcinoembryonic antigen family. Exp Cell Res 252:243–249

Berinstein NL (2002) Carcinoembryonic antigen as a target for therapeutic anticancer vaccines: a review. J Clin Oncol 20:2197–2207

Birchmeier W, Behrens J (1994) Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim Biophys Acta 1198:11–26

Blau DM, Turbide C, Tremblay M, Olson M, Letourneau S, Michaliszyn E, Jothy S, Holmes KV, Beauchemin N (2001) Targeted disruption of the ceacam1 (mhvr) gene leads to reduced susceptibility of mice to mouse hepatitis virus infection. J Virol 75:8173–8186

Boehm MK, Mayans MO, Thornton JD, Begent RHJ, Keep PA, Perkins SJ (1996) Extended glycoprotein structure of the seven domains in human carcinoembryonic antigen by X-ray and neutron solution scattering and an automated curve fitting procedure: implications for cellular adhesion. J Mol Biol 259:718–736

Booth JW, Telio D, Liao EH, McCaw SE, Matsuo T, Grinstein S, Gray-Owen SD (2003) Phosphatidylinositol 3-kinases in CEACAM-mediated internalization of Neisseria gonorrhoeae. J Biol Chem 278:14037–14045

Bos MP, Kuroki M, Krop-Watorek A, Hogan D, Belland RJ (1998) CD66 receptor specificity exhibited by neisserial Opa variants is controlled by protein determinants in CD66 N-domains. Proc Natl Acad Sci U S A 95:9584–9589

Botling J, Oberg F, Nilsson K (1995) CD49f (alpha 6 integrin) and CD66a (BGP) are specifically induced by retinoids during human monocytic differentiation. Leukemia 9:2034–2041

Boulton IC, Gray-Owen SD (2002) Neisserial binding to CEACAM1 arrests the activation and proliferation of CD4+ T lymphocytes. Nat Immunol 3:229–236

Bracke ME, Van Roy FM, Mareel MM (1996) The E-cadherin/catenin complex in invasion and metastasis. Curr Top Microbiol Immunol 213:123–161

Bradbury J (2002) Neisseria gonorrhoeae evades host immunity by switching off T lymphocytes. Lancet 359:681

Brümmendorf T, Lemmon V (2001) Immunoglobulin superfamily receptors: *cis*-interactions, intracellular adapters and alternative splicing regulate adhesion. Curr Opin Cell Biol 13:611–618

Brümmer J, Neumaier M, Göpfert C, Wagener C (1995) Association of pp60c-src with biliary glycoprotein (CD66a), an adhesion molecule of the carcinoembryonic antigen family downregulated in colorectal carcinomas. Oncogene 11:1649–1655

Brümmer J, Ebrahimnejad A, Flayeh R, Schumacher U, Löning T, Bamberger AM, Wagener C (2001) *cis* Interaction of the cell adhesion molecule CEACAM1 with integrin beta(3). Am J Pathol 159:537–546

Budt M, Cichocka I, Reutter W, Lucka L (2002) Clustering-induced signaling of CEACAM1 in PC12 cells. Biol Chem 383:803–812

Bunjes D, Buchmann I, Duncker C, Seitz U, Kotzerke J, Wiesneth M, Dohr D, Stefanic M, Buck A, Harsdorf SV, Glatting G, Grimminger W, Karakas T, Munzert G, Dohner H, Bergmann L, Reske SN (2001) Rhenium 188-labeled anti-CD66 (a, b, c, e) monoclonal

antibody to intensify the conditioning regimen prior to stem cell transplantation for patients with high-risk acute myeloid leukemia or myelodysplastic syndrome: results of a phase I-II study. Blood 98:565–572

Busch C, Hanssen TA, Wagener C, Öbrink B (2002) Down-regulation of CEACAM1 in human prostate cancer: correlation with loss of cell polarity, increased proliferation rate, and Gleason grade 3 to 4 transition. Hum Pathol 33:290–298

Byzova TV, Goldman CK, Pampori N, Thomas KA, Bett A, Shattil SJ, Plow EF (2000) A mechanism for modulation of cellular responses to VEGF: activation of the integrins. Mol Cell 6:851–860

Cambier JC (1995) Antigen and Fc receptor signaling. The awesome power of the immunoreceptor tyrosine-based activation motif (ITAM). J Immunol 155:3281–3285

Carlson TR, Feng Y, Maisonpierre PC, Mrksich M, Morla AO (2001) Direct cell adhesion to the angiopoietins mediated by integrins. J Biol Chem 276:26516–26525

Carmeliet P, Jain RK (2000) Angiogenesis in cancer and other diseases. Nature 407:249–257

Carmeliet P, Moons L, Luttun A, Vincenti V, Compernolle V, De Mol M, Wu Y, Bono F, Devy L, Beck H, Scholz D, Acker T, DiPalma T, Dewerchin M, Noel A, Stalmans I, Barra A, Blacher S, Vandendriessche T, Ponten A, Eriksson U, Plate KH, Foidart JM, Schaper W, Charnock-Jones DS, Hicklin DJ, Herbert JM, Collen D, Persico MG (2001) Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat Med 7:575–583

Cavallaro U, Niedermeyer J, Fuxa M, Christofori G (2001) N-CAM modulates tumour-cell adhesion to matrix by inducing FGF-receptor signalling. Nat Cell Biol 3:650–657

Chen CJ, Lin TT, Shively JE (1996) Role of interferon regulatory factor-1 in the induction of biliary glycoprotein (cell CAM-1) by interferon-gamma. J Biol Chem 271:28181–28188

Chen T, Belland RJ, Wilson J, Swanson J (1995) Adherence of pilus- Opa+ gonococci to epithelial cells in vitro involves heparan sulfate. J Exp Med 182:511–517

Chen T, Grunert F, Medina-Marino A, Gotschlich EC (1997) Several carcinoembryonic antigens (CD66) serve as receptors for gonococcal opacity proteins. J Exp Med 185:1557–1564

Chen T, Bolland S, Chen I, Parker J, Pantelic M, Grunert F, Zimmermann W (2001) The CGM1a (CEACAM3/CD66d)-mediated phagocytotic pathway of Neisseria gonorrhea expressing opacity proteins is also the pathway to cell death. J Biol Chem 276:17413–17419

Cheung PH, Luo W, Qiu Y, Zhang X, Earley K, Millirons P, Lin SH (1993) Structure and function of C-CAM1. The first immunoglobulin domain is required for intercellular adhesion. J Biol Chem 268:24303–24310

Choice CV, Howard MJ, Poy MN, Hankin MH, Najjar SM (1998) Insulin stimulates pp120 endocytosis in cells co-expressing insulin receptors. J Biol Chem 273:22194–22200

Chothia C, Gelfand I, Kister A (1998) Structural determinants in the sequences of immunoglobulin variable domain. J Mol Biol 278:457–479

Compton SR, Barthold SW, Smith AL (1993) The cellular and molecular pathogenesis of coronaviruses. Lab Anim Sci 43:15–28

Conway EM, Collen D, Carmeliet P (2001) Molecular mechanisms of blood vessel growth. Cardiovasc Res 49:507–521

Cournoyer D, Beauchemin N, Boucher D, Benchimol S, Fuks A, Stanners CP (1988) Transcription of genes of the carcinoembryonic antigen family in malignant and nonmalignant human tissues. Cancer Res 48:3153–3157

Da Silva-Azevedo L, Reutter W (1999) The long isoform of the cell adhesion molecule C-CAM binds to actin. Biochem Biophys Res Commun 256:404–408

Daniels E, Letourneau S, Turbide C, Kuprina N, Rudinskaya T, Yazova AC, Holmes KV, Dveksler GS, Beauchemin N (1996) Biliary glycoprotein 1 expression during embryogenesis: correlation with events of epithelial differentiation, mesenchymal-epithelial interactions, absorption, and myogenesis. Dev Dyn 206:272–290

Dehio C, Gray-Owen SD, Meyer TF (1998) The role of neisserial Opa proteins in interactions with host cells. Trends Microbiol 6:489–495

Dejana E, Spagnuolo R, Bazzoni G (2001) Interendothelial junctions and their role in the control of angiogenesis, vascular permeability and leukocyte transmigration. Thromb Haemost 86:308–315

Dejmek A, Hjerpe A (1994) Carcinoembryonic antigen-like reactivity in malignant mesothelioma. A comparison between different commercially available antibodies. Cancer 73:464–469

Donda A, Mori L, Shamshiev A, Carena I, Mottet C, Heim MH, Beglinger C, Grunert F, Rochlitz C, Terracciano L, Jantscheff P, De Libero G (2000) Locally inducible CD66a (CEACAM1) as an amplifier of the human intestinal T cell response. Eur J Immunol 30:2593–2603

Draberova L, Cerna H, Brodska H, Boubelik M, Watt SM, Stanners CP, Draber P (2000) Soluble isoforms of CEACAM1 containing the A2 domain: increased serum levels in patients with obstructive jaundice and differences in 3-fucosyl-N-acetyl-lactosamine moiety. Immunology 101:279–287

Drzeniek Z, Lamerz R, Fenger U, Wagener C, Haubeck H-D (1991) Identification of membrane antigens in granulocytes and colonic carcinoma cells by a monoclonal antibody specific for biliary glycoprotein, a member of the carcinoembryonic antigen family. Cancer Lett 56:173–179

Dveksler GS, Pensiero MN, Cardellichio CB, Williams RK, Jiang GS, Holmes KV, Dieffenbach CW (1991) Cloning of the mouse hepatitis virus (MHV) receptor: expression in human and hamster cell lines confers susceptibility to MHV. J Virol 65:6881–6891

Dveksler GS, Dieffenbach CW, Cardellichio CB, McCuaig K, Pensiero MN, Jiang GS, Beauchemin N, Holmes KV (1993a) Several members of the mouse carcinoembryonic antigen-related glycoprotein family are functional receptors for the coronavirus mouse hepatitis virus-A59. J Virol 67:1–8

Dveksler GS, Pensiero MN, Dieffenbach CW, Cardellichio CB, Basile AA, Elia PE, Holmes KV (1993b) Mouse hepatitis virus strain A59 and blocking antireceptor monoclonal antibody bind to the N-terminal domain of cellular receptor. Proc Natl Acad Sci U S A 90:1716–1720

Dveksler GS, Basile AA, Cardellichio CB, Holmes KV (1995) Mouse hepatitis virus receptor activities of an MHVR/mph chimera and MHVR mutants lacking N-linked glycosylation of the N-terminal domain. J Virol 69:543–546

Eades-Perner A-M, van der Putten H, Hirth A, Thompson J, Neumaier M, von Kleist S, Zimmermann W (1994) Mice transgenic for the human carcinoembryonic antigen gene maintain its spatiotemporal expression pattern. Cancer Res 54:4169–4176

Ebrahimnejad A, Flayeh R, Unteregger G, Wagener C, Brümmer J (2000) Cell adhesion molecule CEACAM1 associates with paxillin in granulocytes and epithelial and endothelial cells. Exp Cell Res 260:365–373

Edlund M, Gaardsvoll H, Bock E, Öbrink B (1993) Different isoforms of stock-specific variants of the cell adhesion molecule C-CAM (cell CAM 105) in rat liver. Eur J Biochem 213:1109–1116

Edlund M, Blikstad I, Öbrink B (1996) Calmodulin binds to specific sequences in the cytoplasmic domain of C-CAM and down-regulates C-CAM self-association. J Biol Chem 271:1393–1399

Edlund M, Wikström K, Toomik R, Ek P, Öbrink B (1998) Characterization of protein kinase C-mediated phosphorylation of the short cytoplasmic domain isoform of C-CAM. FEBS Lett 425:166–170

Elghetany MT (2002) Surface antigen changes during normal neutrophilic development: a critical review. Blood Cells Mol Dis 28:260–274

Ergün S, Kilic N, Ziegeler G, Hansen A, Nollau P, Götze J, Wurmbach JH, Horst A, Weil J, Fernando M, Wagener C (2000) CEA-related cell adhesion molecule 1: a potent angiogenic factor and a major effector of vascular endothelial growth factor. Mol Cell 5:311–320

Eshdat Y, Ofek I, Yashouv-Gan Y, Sharon N, Mirelman D (1978) Isolation of a mannose-specific lectin from *E. coli* and its rule in the adherence of the bacteria to epithelial cells. Biochem Biophys Res Commun 85:1551–1559

Estrera VT, Chen DT, Luo W, Hixson DC, Lin SH (2001) Signal transduction by the CEACAM1 tumor suppressor. Phosphorylation of serine 503 is required for growth-inhibitory activity. J Biol Chem 276:15547–15553

Famiglietti J, Sun J, DeLisser HM, Albelda SM (1997) Tyrosine residue in exon 14 of the cytoplasmic domain of platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) regulates ligand binding specificity. J Cell Biol 138:1425–1435

Ferrero E, Ferrero ME, Pardi R, Zocchi MR (1995) The platelet endothelial cell adhesion molecule-1 (PECAM1) contributes to endothelial barrier function. FEBS Lett 374:323–326

Feuk-Lagerstedt E, Jordan ET, Leffler H, Dahlgren C, Karlsson A (1999) Identification of CD66a and CD66b as the major galectin-3 receptor candidates in human neutrophils. J Immunol 163:5592–5598

Folkman J, ĎAmore PA (1996) Blood vessel formation: what is the molecular basis? Cell 87:1153–1155

Fournes B, Sadekova S, Turbide C, Letourneau S, Beauchemin N (2001) The CEACAM1-L Ser503 residue is crucial for inhibition of colon cancer cell tumorigenicity. Oncogene 20:219–230

Fournes B, Farrah J, Olson M, Lamarche-Vane N, Beauchemin N (2003) Distinct Rho GTPase activities regulate epithelial cell localization of the adhesion molecule CEACAM1: involvement of the CEACAM1 transmembrane domain. Mol Cell Biol 23:7291–7304

Frängsmyr L, Baranov V, Prall F, Yeung MM, Wagener C, Hammarström S (1995) Cell- and region-specific expression of biliary glycoprotein and its messenger RNA in normal human colonic mucosa. Cancer Res 55:2963–2967

Frängsmyr L, Baranov V, Hammarström S (1999) Four carcinoembryonic antigen subfamily members, CEA, NCA, BGP and CGM2, selectively expressed in the normal human colonic epithelium, are integral components of the fuzzy coat. Tumour Biol 20:277–292

Fukushima K, Ohkura T, Kanai M, Kuroki M, Matsuoka Y, Kobata A, Yamashita K (1995) Carbohydrate structures of a normal counterpart of the carcinoembryonic antigen produced by colon epithelial cells of normal adults. Glycobiology 5:105–115

Gagneten S, Scanga CA, Dveksler GS, Beauchemin N, Percy D, Holmes KV (1996) Attachment glycoproteins and receptor specificity of rat coronaviruses. Lab Anim Sci 46:159–166

Gbarah A, Mhashilkar AM, Boner G, Sharon N (1989) Involvement of protein kinase C in activation of human granulocytes and peritoneal macrophages by type 1 fimbriated (mannose specific) *Escherichia coli*. Biochem Biophys Res Commun 165:1243–1249

Gehling UM, Ergün S, Schumacher U, Wagener C, Pantel K, Otte M, Schuch G, Schafhausen P, Mende T, Kilic N, Kluge K, Schäfer B, Hossfeld DK, Fiedler W (2000) In vitro differentiation of endothelial cells from AC133-positive progenitor cells. Blood 95:3106–3112

Gold P, Freedman SO (1965) Specific carcinoembryonic antigens of the human digestive system. J Exp Med 122:467–481

Grassme H, Gulbins E, Brenner B, Ferlinz K, Sandhoff K, Harzer K, Lang F, Meyer TF (1997) Acidic sphingomyelinase mediates entry of N. gonorrhoeae into nonphagocytic cells. Cell 91:605–615

Gray-Owen SD, Dehio C, Haude A, Grunert F, Meyer TF (1997a) CD66 carcinoembryonic antigens mediate interactions between Opa-expressing Neisseria gonorrhoeae and human polymorphonuclear phagocytes. EMBO J 16:3435–3445

Gray-Owen SD, Lorenzen DR, Haude A, Meyer TF, Dehio C (1997b) Differential Opa specificities for CD66 receptors influence tissue interactions and cellular response to Neisseria gonorrhoeae. Mol Microbiol 26:971–980

Greicius G, Severinson E, Beauchemin N, Öbrink B, Singer BB (2003) CEACAM1 is a potent regulator of B cell receptor complex-induced activation. J Leukoc Biol 74:126–134

Hajra KM, Fearon ER (2002) Cadherin and catenin alterations in human cancer. Genes Chromosomes Cancer 34:255–268

Hammarström S (1999) The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol 9:67–81

Hammarström S, Baranov V (2001) Is there a role for CEA in innate immunity in the colon? Trends Microbiol 9:119–125

Han E, Phan D, Lo P, Poy MN, Behringer R, Najjar SM, Lin SH (2001) Differences in tissue-specific and embryonic expression of mouse Ceacam1 and Ceacam2 genes. Biochem J 355:417–423

Hanahan D, Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during angiogenesis. Cell 86:353–364

Hanenberg H, Baumann M, Quentin I, Nagel G, Grosse-Wilde H, von Kleist S, Gobel U, Burdach S, Grunert F (1994) Expression of the CEA gene family members NCA-50/90 and NCA-160 (CD66) in childhood acute lymphoblastic leukemias (ALLs) and in cell lines of B-cell origin. Leukemia 8:2127–2133

Hansson M, Blikstad I, Öbrink B (1989) Cell-surface location and molecular properties of cell-CAM 105 in intestinal epithelial cells. Exp Cell Res 181:63–74

Harpaz Y, Chothia C (1994) Many of the immunoglobulin superfamily domains in cell adhesion molecules and surface receptors belong to a new structural set which is close to that containing variable domains. J Mol Biol 238:528–539

Hauck W, Stanners CP (1991) Control of carcinoembryonic antigen gene family expression in a differentiating colon carcinoma cell line, Caco-2. Cancer Res 51:3526–3533

Hauck CR, Meyer TF, Lang F, Gulbins E (1998) CD66-mediated phagocytosis of opa52 Neisseria gonorrhoeae requires a src-like tyrosine kinase- and rac-1-dependent signalling pathway. EMBO J 17:443–454

Hauck CR, Gulbins E, Lang F, Meyer TF (1999) Tyrosine phosphatase SHP-1 is involved in CD66-mediated phagocytosis of Opa52-expressing Neisseria gonorrhoeae. Infect Immun 67:5490–5494

Hauck CR, Grassme H, Bock J, Jendrossek V, Ferlinz K, Meyer TF, Gulbins E (2000) Acid sphingomyelinase is involved in CEACAM receptor-mediated phagocytosis of Neisseria gonorrhoeae. FEBS Lett 478:260–266

Hauck W, Nedellec P, Turbide C, Stanners CP, Barnett TR, Beauchemin N (1994) Transcriptional control of the human biliary glycoprotein gene, a CEA gene family member down-regulated in colorectal carcinomas. Eur J Biochem 223:529–541

Hazan RB, Norton L (1998) The epidermal growth factor receptor modulates the interaction of E-cadherin with the actin cytoskeleton. J Biol Chem 273:9078–9084

Hill DJ, Toleman MA, Evans DJ, Villullas S, Van Alphen L, Virji M (2001) The variable P5 proteins of typeable and non-typeable Haemophilus influenzae target human CEACAM1. Mol Microbiol 39:850–862

Hinoda Y, Imai K, Nakagawa N, Ibayashi Y, Nakano T, Paxton RJ, Shively JE, Yachi A (1990) Transcription of biliary glycoprotein I gene in malignant and non-malignant human liver tissues. Int J Cancer 45:875–878

Hixson DC, McEntire KD, Öbrink B (1985) Alterations in the expression of a hepatocyte cell adhesion molecule by transplantable rat hepatocellular carcinomas. Cancer Res 45:3742–3749

Honig M, Peter HH, Jantscheff P, Grunert F (1999) Synovial PMN show a coordinated upregulation of CD66 molecules. J Leukoc Biol 66:429–436

Hornig C, Barleon B, Ahmad S, Vuorela P, Ahmed A, Weich HA (2000) Release and complex formation of soluble VEGFR-1 from endothelial cells and biological fluids. Lab Invest 80:443–454

Houde C, Roy S, Leung N, Nicholson DW, Beauchemin N (2003) The cell adhesion molecule CEACAM1-L is a substrate of caspase-3-mediated cleavage in apoptotic mouse intestinal cells. J Biol Chem 278:16929–16935

Hsieh JT, Luo W, Song W, Wang Y, Kleinerman DI, Van NT, Lin SH (1995) Tumor suppressive role of an androgen-regulated epithelial cell adhesion molecule (C-CAM) in prostate carcinoma cell revealed by sense and antisense approaches. Cancer Res 55:190–197

Huang J, Simpson JF, Glackin C, Riethorf L, Wagener C, Shively JE (1998) Expression of biliary glycoprotein (CD66a) in normal and malignant breast epithelial cells. Anticancer Res 18:3203–3212

Huang JQ, Turbide C, Daniels E, Jothy S, Beauchemin N (1990) Spatiotemporal expression of murine carcinoembryonic antigen (CEA) gene family members during mouse embryogenesis. Development 110:573–588

Huber M, Izzi L, Grondin P, Houde C, Kunath T, Veillette A, Beauchemin N (1999) The carboxyl-terminal region of biliary glycoprotein controls its tyrosine phosphorylation and association with protein-tyrosine phosphatases SHP-1 and SHP-2 in epithelial cells. J Biol Chem 274:335–344

Huber M, Izzi L, Grondin P, Houde C, Kunath T, Veillette A, Beauchemin N (1999) The carboxy-terminal region of biliary glycoprotein controls its tyrosine phosphorylation and association with protein-tyrosine phosphatases SHP-1 and SHP-2 in epithelial cells. J Biol Chem 274:335–344

Hunter I, Sawa H, Edlund M, Öbrink B (1996) Evidence for regulated dimerization of cell–cell adhesion molecule (C-CAM) in epithelial cells. Biochem J 320:847–853

Hunter I, Sigmundsson K, Beauchemin N, Öbrink B (1998) The cell adhesion molecule C-CAM is a substrate for tissue transglutaminase. FEBS Lett 425:141–144

Ilantzis C, Jothy S, Alpert LC, Draber P, Stanners CP (1997) Cell-surface levels of human carcinoembryonic antigen are inversely correlated with colonocyte differentiation in colon carcinogenesis. Lab Invest 76:703–716

Izzi L, Turbide C, Houde C, Kunath T, Beauchemin N (1999) *cis*-Determinants in the cytoplasmic domain of CEACAM1 responsible for its tumor inhibitory function. Oncogene 18:5563–5572

Jothy S, Yuan SY, Shirota K (1993) Transcription of carcinoembryonic antigen in normal colon and colon carcinoma. In situ hybridization study and implication for a new in vivo functional model. Am J Pathol 143:250–257

Juliano RL (2002) Signal transduction by cell adhesion receptors and the cytoskeleton: functions of integrins, cadherins, selectins, and immunoglobulin-superfamily members. Annu Rev Pharmacol Toxicol 42:283–323

Kammerer R, Hahn S, Singer BB, Luo JS, von Kleist S (1998) Biliary glycoprotein (CD66a), a cell adhesion molecule of the immunoglobulin superfamily, on human lymphocytes: structure, expression and involvement in T cell activation. Eur J Immunol 28:3664–3674

Kammerer R, Stober D, Singer BB, Öbrink B, Reimann J (2001) Carcinoembryonic antigen-related cell adhesion molecule 1 on murine dendritic cells is a potent regulator of T cell stimulation. J Immunol 166:6537–6544

Kannicht C, Lucka L, Nuck R, Reutter W, Gohlke M (1999) N-glycosylation of the carcinoembryonic antigen related cell adhesion molecule, C-CAM, from rat liver: detection of oversialylated bi- and triantennary structures. Glycobiology 9:897–906

Kerr MA, Stocks SC (1992) The role of CD15-(Le(X))-related carbohydrates in neutrophil adhesion. Histochem J 24:811–826

Khan WN, Hammarström S, Ramos T (1993) Expression of antigens of the carcinoembryonic antigen family on B cell lymphomas and Epstein-Barr virus immortalized B cell lines. Int Immunol 5:265–270

Kim J, Kaye FJ, Henslee JG, Shively JE, Park JG, Lai SL, Linnoila RI, Mulshine JL, Gazdar AF (1992) Expression of carcinoembryonic antigen and related genes in lung and gastrointestinal cancers. Int J Cancer 52:718–725

Kinugasa T, Kuroki M, Takeo H, Matsuo Y, Ohshima K, Yamashita Y, Shirakusa T, Matsuoka Y (1998) Expression of four CEA family antigens (CEA, NCA, BGP and CGM2) in normal and cancerous gastric epithelial cells: up-regulation of BGP and CGM2 in carcinomas. Int J Cancer 76:148–153

Kirshner J, Chen CJ, Liu P, Huang J, Shively JE (2003a) CEACAM1-4S, a cell–cell adhesion molecule, mediates apoptosis and reverts mammary carcinoma cells to a normal morphogenic phenotype in a 3D culture. Proc Natl Acad Sci U S A 100:521–526

Kirshner J, Schumann D, Shively JE (2003b) CEACAM1, a cell–cell adhesion molecule, directly associates with annexin II in a 3D model of mammary morphogenesis. J Biol Chem M309115200

Klein ML, McGhee SA, Baranian J, Stevens L, Hefta SA (1996) Role of nonspecific cross-reacting antigen, a CD66 cluster antigen, in activation of human granulocytes. Infect Immun 64:4574–4579

Kleinerman DI, Troncoso P, Lin SH, Pisters LL, Sherwood ER, Brooks T, von Eschenbach AC, Hsieh JT (1995a) Consistent expression of an epithelial cell adhesion molecule (C-CAM) during human prostate development and loss of expression in prostate cancer: implication as a tumor suppressor. Cancer Res 55:1215–1220

Kleinerman DI, Zhang WW, Lin SH, Nguyen TV, von Eschenbach AC, Hsieh JT (1995b) Application of a tumor suppressor (C-CAM1)-expressing recombinant adenovirus in androgen-independent human prostate cancer therapy: a preclinical study. Cancer Res 55:2831–2836

Kleinerman DI, Dinney CP, Zhang WW, Lin SH, Van NT, Hsieh JT (1996) Suppression of human bladder cancer growth by increased expression of C-CAM1 gene in an orthotopic model. Cancer Res 56:3431–3435

Kodera Y, Isobe K, Yamauchi M, Satta T, Hasegawa T, Oikawa S, Kondoh K, Akiyama S, Itoh K, Nakashima I, et al (1993) Expression of carcinoembryonic antigen (CEA) and nonspecific crossreacting antigen (NCA) in gastrointestinal cancer; the correlation with degree of differentiation. Br J Cancer 68:130–136

Kondo Y, Hinoda Y, Akashi H, Sakamoto H, Itoh F, Hirata K, Kuroki M, Imai K (2001) Measurement of circulating biliary glycoprotein (CD66a) in liver diseases. J Gastroenterol 36:470–475

Kostrewa D, Brockhaus M, D'Arcy A, Dale GE, Nelboeck P, Schmid G, Mueller F, Bazzoni G, Dejana E, Bartfai T, Winkler FK, Hennig M (2001) X-ray structure of junctional adhesion molecule: structural basis for homophilic adhesion via a novel dimerization motif. EMBO J 20:4391–4398

Kunath T, Ordonez-Garcia C, Turbide C, Beauchemin N (1995) Inhibition of colonic tumor cell growth by biliary glycoprotein. Oncogene 11:2375–2382

Kupsch EM, Knepper B, Kuroki T, Heuer I, Meyer TF (1993) Variable opacity (Opa) outer membrane proteins account for the cell tropisms displayed by Neisseria gonorrhoeae for human leukocytes and epithelial cells. EMBO J 12:641–650

Kuroki M, Yamanaka T, Matsuo Y, Oikawa S, Nakazato H, Matsuoka Y (1995) Immunochemical analysis of carcinoembryonic antigen (CEA)-related antigens differentially localized in intracellular granules of human neutrophils. Immunol Invest 24:829–843

Laack E, Nikbakht H, Peters A, Kugler C, Jasiewicz Y, Edler L, Brümmer J, Schumacher U, Hossfeld DK (2002) Expression of CEACAM1 in adenocarcinoma of the lung: a factor of independent prognostic significance. J Clin Oncol 20:4279–4284

Leslie KK, Watanabe S, Lei KJ, Chou DY, Plouzek CA, Deng HC, Torres J, Chou JY (1990) Linkage of two human pregnancy-specific beta 1-glycoprotein genes: one is associated with hydatidiform mole. Proc Natl Acad Sci U S A 87:5822–5826

Leusch H-G, Hefta SA, Drzeniek Z, Hummel K, Markos-Pusztai Z, Wagener C (1990) *Escherichia coli* of human origin binds to carcinoembryonic antigen (CEA) and nonspecific crossreacting antigen (NCA). FEBS Lett 261:405–409

Leusch H-G, Drzeniek Z, Hefta SA, Markos-Pusztai Z, Wagener C (1991a) The putative role of members of the CEA-gene family (CEA, NCA, and BGP) as ligands for the bacterial colonization of different human epithelial tissues. Zentralbl Bakteriol 275:118–122

Leusch H-G, Drzeniek Z, Markos-Pusztai Z, Wagener C (1991b) Binding of *E. coli* and Salmonella strains to members of the CEA family: differential binding inhibition by aromatic α-glycosides of mannose. Infect Immun 59:2051–2057

Lin SH, Luo W, Earley K, Cheung P, Hixson DC (1995) Structure and function of C-CAM1: effects of the cytoplasmic domain on cell aggregation. Biochem J 311:239–245

Long EO (1999) Regulation of immune responses through inhibitory receptors. Annu Rev Immunol 17:875–904

Lucka L, Sel S, Danker K, Horstkorte R, Reutter W (1998) Carcinoembryonic antigen-related cell–cell adhesion molecule C-CAM is greatly increased in serum and urine of rats with liver diseases. FEBS Lett 438:37–40

Lüning C, Wroblewski J, Öbrink B, Hammarström L, Rozell B (1995) C-CAM expression in odontogenesis and tooth eruption. Connect Tissue Res 32:201–207

Luo W, Wood CG, Earley K, Hung M-C, Lin S-H (1997) Suppression of tumorigenicity of breast cancer cells by an epithelial cell adhesion molecule (C-CAM-1): the adhesion and growth suppression are mediated by different domains. Oncogene 14:1697–1704

Luo W, Tapolsky M, Earley K, Wood CG, Wilson DR, Logothetis CJ, Lin SH (1999) Tumor-suppressive activity of CD66a in prostate cancer. Cancer Gene Ther 6:313–321

Mackay DJ, Hall A (1998) Rho GTPases. J Biol Chem 273:20685–20688

Mahrenholz AM, Yeh CH, Shively JE, Hefta SA (1993) Microsequence and mass spectral analysis of nonspecific cross-reacting antigen 160, a CD15-positive neutrophil membrane glycoprotein. Biol Chem 268:13015–13018

Majuri ML, Hakkarainen M, Paavonen T, Renkonen R (1994) Carcinoembryonic antigen is expressed on endothelial cells. A putative mediator of tumor cell extravasation and metastasis. Apmis 102:432–438

Makarovskiy AN, Pu YS, Lo P, Earley K, Paglia M, Hixson DC, Lin SH (1999) Expression and androgen regulation of C-CAM cell adhesion molecule isoforms in rat dorsal and ventral prostate. Oncogene 18:3252–3260

Mandelboim O, Malik P, Davis DM, Jo CH, Boyson JE, Strominger JL (1999) Human CD16 as a lysis receptor mediating direct natural killer cell cytotoxicity. Proc Natl Acad Sci U S A 96:5640–5644

Margolis RN, Schell MJ, Taylor SI, Hubbard AL (1990) Hepatocyte plasma membrane ECTO-ATPase (pp120/HA4) is a substrate for tyrosine kinase activity of the insulin receptor. Biochem Biophys Res Commun 166:562–566

Markel G, Lieberman N, Katz G, Arnon TI, Lotem M, Drize O, Blumberg RS, Bar-Haim E, Mader R, Eisenbach L, Mandelboim O (2002a) CD66a interactions between human melanoma and NK cells: a novel class I MHC-independent inhibitory mechanism of cytotoxicity. J Immunol 168:2803–2810

Markel G, Wolf D, Hanna J, Gazit R, Goldman-Wohl D, Lavy Y, Yagel S, Mandelboim O (2002b) Pivotal role of CEACAM1 protein in the inhibition of activated decidual lymphocyte functions. J Clin Invest 110:943–953

Markel G, Mussaffi H, Ling KL, Salio M, Gadola S, Steuer G, Blau H, Achdout H, De Miguel M, Gonen-Gross T, Hanna J, Arnon TI, Qimron U, Volovitz I, Eisenbach L, Blumberg RS, Porgador A, Cerundolo V, Mandelboim O (2003) The mechanisms controlling NK cell autoreactivity in TAP2-deficient patients. Blood 103:1770–1778

Martin M, Romero X, de la Fuente MA, Tovar V, Zapater N, Esplugues E, Pizcueta P, Bosch J, Engel P (2001) CD84 functions as a homophilic adhesion molecule and enhances IFN-gamma secretion: adhesion is mediated by Ig-like domain 1. J Immunol 167:3668–3676

McCaw SE, Schneider J, Liao EH, Zimmermann W, Gray-Owen SD (2003) Immunoreceptor tyrosine-based activation motif phosphorylation during engulfment of Neisseria gonorrhoeae by the neutrophil-restricted CEACAM3 (CD66d) receptor. Mol Microbiol 49:623–637

McCuaig K, Turbide C, Beauchemin N (1992) mmCGM1a: a mouse carcinoembryonic antigen gene family member, generated by alternative splicing, functions as an adhesion molecule. Cell Growth Differ 3:165–174

McCuaig K, Rosenberg M, Nedellec P, Turbide C, Beauchemin N (1993) Expression of the Bgp gene and characterization of mouse colon biliary glycoprotein isoforms. Gene 127:173–183

McEver RP (2002) Selectins: lectins that initiate cell adhesion under flow. Curr Opin Cell Biol 14:581–586

McGee ZA, Stephens DS, Hoffman LH, Schlech WF, 3rd Horn RG (1983) Mechanisms of mucosal invasion by pathogenic Neisseria. Rev Infect Dis 5 Suppl 4:S708–714

Metze D, Soyer H-P, Zelger B, Neumaier M, Grunert F, Hartig C, Amann U, Bhardwaj R, Wagener C, Luger TA (1996) Expression of a glycoprotein of the carcinoembryonic antigen family in normal and neoplastic sebaceous glands. J Am Acad Dermatol 134:735–744

Meyer TF, van Putten JP (1989) Genetic mechanisms and biological implications of phase variation in pathogenic neisseriae. Clin Microbiol Rev 2 Suppl:S139–145

Moffett-King A (2002) Natural killer cells and pregnancy. Nat Rev Immunol 2:656–663

Möller MJ, Kammerer R, Grunert F, von Kleist S (1996) Biliary glycoprotein (BGP) expression on T cells and on a natural-killer-cell sub-population. Int J Cancer 65:740–745

Morales VM, Christ A, Watt SM, Kim HS, Johnson KW, Utku N, Texieira AM, Mizoguchi A, Mizoguchi E, Russell GJ, Russell SE, Bhan AK, Freeman GJ, Blumberg RS (1999) Regulation of human intestinal intraepithelial lymphocyte cytolytic function by biliary glycoprotein (CD66a). J Immunol 163:1363–1370

Muenzner P, Naumann M, Meyer TF, Gray-Owen SD (2001) Pathogenic Neisseria trigger expression of their carcinoembryonic antigen-related cellular adhesion molecule 1 (CEACAM1; previously CD66a) receptor on primary endothelial cells by activating the immediate early response transcription factor, nuclear factor-kappaB. J Biol Chem 276:24331–24340

Muenzner P, Billker O, Meyer TF, Naumann M (2002) Nuclear factor-kappa B directs carcinoembryonic antigen-related cellular adhesion molecule 1 receptor expression in Neisseria gonorrhoeae-infected epithelial cells. J Biol Chem 277:7438–7446

Nagar B, Overduin M, Ikura M, Rini JM (1996) Structural basis of calcium-induced E-cadherin rigidification and dimerization. Nature 380:360–364

Nair KS, Zingde SM (2001) Adhesion of neutrophils to fibronectin: role of the cd66 antigens. Cell Immunol 208:96–106

Najjar SM (2002) Regulation of insulin action by CEACAM1. Trends Endocrinol Metab 13:240–245

Najjar SM, Accili D, Philippe N, Jernberg J, Margolis R, Taylor SI (1993) pp120/ecto-ATPase, an endogenous substrate of the insulin receptor tyrosine kinase, is expressed as two variably spliced isoforms. J Biol Chem 268:1201–1206

Najjar SM, Philippe N, Suzuki Y, Ignacio GA, Formisano P, Accili D, Taylor SI (1995) Insulin-stimulated phosphorylation of recombinant pp120/HA4, an endogenous substrate of the insulin receptor tyrosine kinase. Biochemistry 34:9341–9349

Najjar SM, Boisclair YR, Nabih ZT, Philippe N, Imai Y, Suzuki Y, Suh DS, Ooi GT (1996) Cloning and characterization of a functional promoter of the rat pp120 gene, encoding a substrate of the insulin receptor tyrosine kinase. J Biol Chem 271:8809–8817

Najjar SM, Blakesley VA, Li Calzi S, Kato H, LeRoith D, Choice CV (1997) Differential phosphorylation of pp120 by insulin and insulin-like growth factor-1 receptors: role for the C-terminal domain of the beta-subunit. Biochemistry 36:6827–6834

Najjar SM, Choice CV, Soni P, Whitman CM, Poy MN (1998) Effect of pp120 on receptor-mediated insulin endocytosis is regulated by the juxtamembrane domain of the insulin receptor. J Biol Chem 273:12923–12928

Nakajima A, Iijima H, Neurath MF, Nagaishi T, Nieuwenhuis EE, Raychowdhury R, Glickman J, Blau DM, Russell S, Holmes KV, Blumberg RS (2002) Activation-induced expression of carcinoembryonic antigen-cell adhesion molecule 1 regulates mouse T lymphocyte function. J Immunol 168:1028–1035

Nap M, Mollgard K, Burtin P, Fleuren GJ (1988) Immunohistochemistry of carcino-embryonic antigen in the embryo, fetus and adult. Tumour Biol 9:145–153

Nap M, Hammarström M-L, Börmer O, Hammarström S, Wagener C, Handt S, Schreyer M, Mach J-P, Buchegger F, von Kleist S, Grunert F, Seguin P, Fuks A, Holm R, Lamerz R (1992) Specificity and affinity of monoclonal antibodies against CEA. Cancer Res 52:2329–2339

Nedellec P, Dveksler GS, Daniels E, Turbide C, Chow B, Basile AA, Holmes KV, Beauchemin N (1994) Bgp2, a new member of the carcinoembryonic antigen-related gene family, encodes an alternative receptor for mouse hepatitis viruses. J Virol 68:4525–4537

Neumaier M, Paululat S, Chan A, Matthaes P, Wagener C (1993) Biliary glycoprotein, a potential human cell adhesion molecule, is down-regulated in colorectal carcinomas. Proc Natl Acad Sci U S A 90:10744–10748

Neumaier M, Paulutat S, Chan A, Mattaes P, Wagener C (1993) BGP, a potential human adhesion molecule, is down-regulated in colorectal carcinoma. Proc Natl Acad Sci U S A 90:10744–10748

Nollau P, Prall F, Helmchen U, Wagener C, Neumaier M (1997) Dysregulation of carcinoembryonic antigen group members CGM2, CD66a (biliary glycoprotein), and nonspecific cross-reacting antigen in colorectal carcinomas. Comparative analysis by northern blot and in situ hybridization. Am J Pathol 151:521–530

Nollau P, Scheller H, Kona-Horstmann M, Rhde S, Hagenmüller F, Wagener C, Neumaier M (1997) Expression of CD66a (human C-CAM) and other members of the carcinoembryonic antigen gene family of adhesion molecules in human colorectal adenomas. Cancer Res 57:2354–2357

Normark S, Albiger B, Jonsson AB (2002) Gonococci cause immunosuppression by engaging a coinhibitory receptor on T lymphocytes. Nat Immunol 3:210–211

Öbrink B (1997) CEA adhesion molecules: multifunctional proteins with signal-regulatory properties. Curr Opin Struct Biol 9:616–626

Öbrink B, Sawa H, Scheffrahn I, Singer BB, Sigmundsson K, Sundberg U, Heymann R, Beauchemin N, Weng G, Ram P, Iyengar R (2002) Computational analysis of isoform-specific signal regulation by CEACAM1-A cell adhesion molecule expressed in PC12 cells. Ann N Y Acad Sci 971:597–607

Ochieng J, Leite-Browning ML, Warfield P (1998) Regulation of cellular adhesion to extracellular matrix proteins by galectin-3. Biochem Biophys Res Commun 246:788–791

Ocklind C, Öbrink B (1982) Intercellular adhesion of rat hepatocytes. Identification of a cell surface glycoprotein involved in the initial adhesion process. J Biol Chem 257:6788–6795

Odin P, Tingström A, Öbrink B (1986) Chemical characterization of cell-CAM 105, a cell-adhesion molecule isolated from rat liver membranes. Biochem J 236:559–568

Ohwada A, Takahashi H, Nagaoka I, Kira S (1994) Biliary glycoprotein mRNA expression is increased in primary lung cancer, especially in squamous cell carcinoma. Am J Respir Cell Mol Biol 11:214–220

Oikawa S, Nakazato H, Kosaki G (1987) Primary structure of human carcinoembryonic antigen (CEA) deduced from cDNA sequence. Biochem Biophys Res Commun 142:511–518

Oikawa S, Inuzuka C, Kuroki M, Arakawa F, Matsuoka Y, Kosaki G, Nakazato H (1991) A specific heterotypic cell adhesion activity between members of carcinoembryonic antigen family, W272 and NCA, is mediated by N-domains. J Biol Chem 266:7995–8001

Olsen A, Teglund S, Nelson D, Gordon L, Copeland A, Georgescu A, Carrano A, Hammarström S (1994) Gene organization of the pregnancy-specific glycoprotein region on human chromosome 19: assembly and analysis of a 700-kb cosmid contig spanning the region. Genomics 23:659–668

Pagel JM, Matthews DC, Appelbaum FR, Bernstein ID, Press OW (2002) The use of radioimmunoconjugates in stem cell transplantation. Bone Marrow Transplant 29:807–816

Pallen CJ, Tong PH (1991) Elevation of membrane tyrosine phosphatase activity in density-dependent growth-arrested fibroblasts. Proc Natl Acad Sci U S A 88:6996–7000

Paxton RJ, Mooser G, Pande H, Lee TD, Shively JE (1987) Sequence analysis of CEA: identification of glycosylation sites and homology with the immunoglobulin supergene family. Proc Natl Acad Sci U S A 84:920–924

Phan D, Sui X, Chen DT, Najjar SM, Jenster G, Lin SH (2001) Androgen regulation of the cell–cell adhesion molecule-1 (Ceacam1) gene. Mol Cell Endocrinol 184:115–123

Phillips SA, Perrotti N, Taylor SI (1987) Rat liver membranes contain a 120 kDa glycoprotein which serves as a substrate for the tyrosine kinases of the receptors for insulin and epidermal growth factor. FEBS Lett 212:141–144

Popp A, Dehio C, Grunert F, Meyer TF, Gray-Owen SD (1999) Molecular analysis of neisserial Opa protein interactions with the CEA family of receptors: identification of determinants contributing to the differential specificities of binding. Cell Microbiol 1:169–181

Poy MN, Ruch RJ, Fernstrom MA, Okabayashi Y, Najjar SM (2002a) Shc and CEACAM1 interact to regulate the mitogenic action of insulin. J Biol Chem 277:1076–1084

Poy MN, Yang Y, Rezaei K, Fernstrom MA, Lee AD, Kido Y, Erickson SK, Najjar SM (2002b) CEACAM1 regulates insulin clearance in liver. Nat Genet 30:270–276

Prall F, Nollau P, Neumaier M, Haubeck H-D, Drzeniek Z, Helmchen U, Löning T, Wagener C (1996) CD66a (BGP), an adhesion molecule of the CEA family, is expressed in epithelium, endothelium and myeloid cells in a wide range of normal human tissues. J Histochem Cytochem 44:35–41

Pu YS, Luo W, Lu HH, Greenberg NM, Lin SH, Gingrich JR (1999) Differential expression of C-CAM cell adhesion molecule in prostate carcinogenesis in a transgenic mouse model. J Urol 162:892–896

Pu YS, Do KA, Luo W, Logothetis CJ, Lin SH (2002) Enhanced suppression of prostate tumor growth by combining C-CAM1 gene therapy and angiogenesis inhibitor TNP-470. Anticancer Drugs 13:743–749

Rao Y, Zhao X, Siu CH (1994) Mechanism of homophilic binding mediated by the neural cell adhesion molecule NCAM. Evidence for isologous interaction. J Biol Chem 269:27540–27548

Rass A, Lüning C, Wroblewski J, Öbrink B (1994) Distribution of C-CAM in developing oral tissues. Anat Embryol (Berl) 190:251–261

Rebstock S, Lucas K, Weiss M, Thompson J, Zimmermann W (1993) Spatiotemporal expression of pregnancy-specific glycoprotein gene rnCGM1 in rat placenta. Dev Dyn 198:171–181

Rees-Jones RW, Taylor SI (1985) An endogenous substrate for the insulin receptor-associated tyrosine kinase. J Biol Chem 260:4461–4467

Riethdorf L, Lisboa BW, Henkel U, Naumann M, Wagener C, Löning T (1997) Differential expression of CD66a (BGP), a cell adhesion molecule of the carcinoembryonic antigen family, in benign, premalignant, and malignant lesions of the human mammary gland. J Histochem Cytochem 45:957–963

Robbins PF, Eggensperger D, Qi CF, Schlom J (1993) Definition of the expression of the human carcinoembryonic antigen and non-specific cross-reacting antigen in human breast and lung carcinomas. Int J Cancer 53:892–897

Robitaille J, Izzi L, Daniels E, Zelus B, Holmes KV, Beauchemin N (1999) Comparison of expression patterns and cell adhesion properties of the mouse biliary glycoproteins Bbgp1 and Bbgp2. Eur J Biochem 264:534–544

Rojas M, Fuks A, Stanners CP (1990) Biliary glycoprotein, a member of the immunoglobulin supergene family functions in vitro as a Ca2+ dependent intercellular adhesion molecule. Cell Growth Differentiation 1:527–533

Rosenberg M, Nedellec P, Jothy S, Fleiszer D, Turbide C, Beauchemin N (1993) The expression of mouse biliary glycorprotein, a carcinoembryonic antigen-related gene, is down-regulated in malignant mouse tissues. Cancer Res 55:4938–4945

Ruchaud-Sparagano MH, Stocks SC, Turley H, Dransfield I (1997) Activation of neutrophil function via CD66: differential effects upon beta 2 integrin mediated adhesion. Br J Haematol 98:612–620

Rudert F, Saunders AM, Rebstock S, Thompson JA, Zimmermann W (1992) Characterization of murine carcinoembryonic antigen gene family members. Mamm Genome 3:262–273

Ruggiero T, Olivero M, Follenzi A, Naldini L, Calogero R, Di Renzo MF (2003) Deletion in a (T)8 microsatellite abrogates expression regulation by 3′-UTR. Nucleic Acids Res 31:6561–6569

Sadekova S, Lamarche-Vane N, Li X, Beauchemin N (2000) The CEACAM1-L glycoprotein associates with the actin cytoskeleton and localizes to cell–cell contact through activation of Rho-like GTPases. Mol Biol Cell 11:65–77

Sanders DS, Kerr MA (1999) Lewis blood group and CEA related antigens; coexpressed cell–cell adhesion molecules with roles in the biological progression and dissemination of tumours. Mol Pathol 52:174–178

Satoh Y, Hayashi T, Takahashi T, Itoh F, Adachi M, Fukui M, Kuroki M, Imai K, Hinoda Y (2002) Expression of CD66a in multiple myeloma. J Clin Lab Anal 16:79–85

Sauter SL, Rutherfurd SM, Wagener C, Shively JE, Hefta SA (1991) Binding of nonspecific cross reacting antigen, a granulocyte membrane glycoprotein, to E.coli expressing type I fimbriae. Infect Immun 59:2485–2493

Sawa H, Kamada K, Sato H, Sendo S, Kondo A, Saito I, Edlund M, Öbrink B (1994) C-CAM expression in the developing rat central nervous system. Brain Res Dev Brain Res 78:35–43

Sawa H, Ukita H, Fukuda M, Kamada H, Saito I, Öbrink B (1997) Spatiotemporal expression of C-CAM in the rat placenta. J Histochem Cytochem 45:1021–1034

Schölzel S, Zimmermann W, Schwarzkopf G, Grunert F, Rogaczewski B, Thompson J (2000) Carcinoembryonic antigen family members CEACAM6 and CEACAM7 are differentially expressed in normal tissues and oppositely deregulated in hyperplastic colorectal polyps and early adenomas. Am J Pathol 156:595–605

Schrewe H, Thompson J, Bona M, Hefta LJ, Maruya A, Hassauer M, Shively JE, von Kleist S, Zimmermann W (1990) Cloning of the complete gene for carcinoembryonic antigen: analysis of its promoter indicates a region conveying cell type-specific expression. Mol Cell Biol 10:2738–2748

Schumann D, Chen CJ, Kaplan B, Shively JE (2001) Carcinoembryonic antigen cell adhesion molecule 1 directly associates with cytoskeleton proteins actin and tropomyosin. J Biol Chem 276:47421–47433

Shapiro L, Fannon AM, Kwong PD, Thompson A, Lehmann MS, Grubel G, Legrand JF, Als-Nielsen J, Colman DR, Hendrickson WA (1995) Structural basis of cell–cell adhesion by cadherins. Nature 374:327–337

Sharon N (1987) Bacterial lectins, cell–cell-recognition and infectious disease. FEBS Lett 217:145–157

Sheahan K, O'Brien MJ, Burke B, Dervan PA, O'Keane JC, Gottlieb LS, Zamcheck N (1990) Differential reactivities of carcinoembryonic antigen (CEA) and CEA-related monoclonal and polyclonal antibodies in common epithelial malignancies. Am J Clin Pathol 94:157–164

Shi ZR, Tacha D, Itzkowitz SH (1994) Monoclonal antibody COL-1 reacts with restricted epitopes on carcinoembryonic antigen: an immunohistochemical study. J Histochem Cytochem 42:1215–1219

Sienel W, Dango S, Woelfle U, Morresi-Hauf A, Wagener C, Brümmer J, Mutschler W, Passlick B, Pantel K (2003) Elevated expression of carcinoembryonic antigen-related cell adhesion molecule 1 promotes progression of non-small cell lung cancer. Clin Cancer Res 9:2260–2266

Sinclair NR (2000) Immunoreceptor tyrosine-based inhibitory motifs on activating molecules. Crit Rev Immunol 20:89–102

Singer BB, Scheffrahn I, Öbrink B (2000) The tumor growth-inhibiting cell adhesion molecule CEACAM1 (C-CAM) is differently expressed in proliferating and quiescent epithelial cells and regulates cell proliferation. Cancer Res 60:1236–1244

Singer BB, Scheffrahn I, Heymann R, Sigmundsson K, Kammerer R, Öbrink B (2002) Carcinoembryonic antigen-related cell adhesion molecule 1 expression and signaling in human, mouse, and rat leukocytes: evidence for replacement of the short cytoplasmic domain isoform by glycosylphosphatidylinositol-linked proteins in human leukocytes. J Immunol 168:5139–5146

Sippel CJ, Fallon RJ, Perlmutter DH (1994) Bile acid efflux mediated by the rat liver canalicular bile acid transport/ecto-ATPase protein requires serine 503 phosphorylation and is regulated by tyrosine 488 phosphorylation. J Biol Chem 269:19539–19545

Skubitz KM, Ducker TP, Goueli SA (1992) CD66 monoclonal antibodies recognize a phosphotyrosine-containing protein bearing a CEA cross-reacting antigen on the surface of human neutrophils. J Immunol 148:852–860

Skubitz KM, Campbell KD, Ahmed K, Skubitz AP (1995) CD66 family members are associated with tyrosine kinase activity in human neutrophils. J Immunol 155:5382–5390

Skubitz KM, Campbell KD, Skubitz AP (1996) CD66a, CD66b, CD66c, and CD66d each independently stimulate neutrophils. J Leukoc Biol 60:106–117

Skubitz KM, Campbell KD, Skubitz AP (2000) Synthetic peptides of CD66a stimulate neutrophil adhesion to endothelial cells. J Immunol 164:4257–4264

Skubitz KM, Campbell KD, Skubitz AP (2001) Synthetic peptides from the N-domains of CEACAMs activate neutrophils. J Pept Res 58:515–526

Soni P, Lakkis M, Poy MN, Fernström MA, Najjar SM (2000) The differential effects of pp120 (Ceacam 1) on the mitogenic action of insulin and insulin-like growth factor 1 are regulated by the nonconserved tyrosine 1316 in the insulin receptor. Mol Cell Biol 20:3896–3905

St Croix B, Sheehan C, Rak JW, Florenes VA, Slingerland JM, Kerbel RS (1998) E-Cadherin-dependent growth suppression is mediated by the cyclin-dependent kinase inhibitor p27(KIP1). J Cell Biol 142:557–571

Stanners C (1998) Cell adhesion and communication mediated by the CEA family: basic and clinical principles. Harwood Academic, Amsterdam

Stern A, Brown M, Nickel P, Meyer TF (1986) Opacity genes in Neisseria gonorrhoeae: control of phase and antigenic variation. Cell 47:61–71

Stocks SC, Kerr MA (1993) Neutrophil NCA-160 (CD66) is the major protein carrier of selectin binding carbohydrate groups LewisX and sialyl lewisX. Biochem Biophys Res Commun 195:478–483

Stocks SC, Kerr MA, Haslett C, Dransfield I (1995) CD66-dependent neutrophil activation: a possible mechanism for vascular selectin-mediated regulation of neutrophil adhesion. J Leukocyte Biol 58:40–48

Stocks SC, Albrechtsen M, Kerr MA (1990) Expression of the CD15 differentiation antigen (3-fucosyl-N-acetyl-lactosamine, LeX) on putative neutrophil adhesion molecules CR3 and NCA-160. Biochem J 268:275–280

Stoffel A, Neumaier M, Gaida FJ, Fenger U, Drzeniek Z, Haubeck HD, Wagener C (1993) Monoclonal, anti-domain and anti-peptide antibodies assign the molecular weight 160,000 granulocyte membrane antigen of the CD66 cluster to a mRNA species encoded by the biliary glycoprotein gene, a member of the carcinoembryonic antigen gene family. J Immunol 150:4978–4984

Streichert T, Ebrahimnejad A, Ganzer S, Flayeh R, Wagener C, Brümmer J (2001) The microbial receptor CEACAM3 is linked to the calprotectin complex in granulocytes. Biochem Biophys Res Commun 289:191–197

Stuart DI, Jones EY (1995) Recognition at the cell surface: recent structural insights. Curr Opin Struct Biol 5:735–743

Sun QH, DeLisser HM, Zukowski MM, Paddock C, Albelda SM, Newman PJ (1996) Individually distinct Ig homology domains in PECAM-1 regulate homophilic binding and modulate receptor affinity. J Biol Chem 271:11090–11098

Sundberg U, Öbrink B (2002) CEACAM1 isoforms with different cytoplasmic domains show different localization, organization and adhesive properties in polarized epithelial cells. J Cell Sci 115:1273–1284

Svalander PC, Odin P, Nilsson BO, Öbrink B (1990) Expression of cellCAM-105 in the apical surface of rat uterine epithelium is controlled by ovarian steroid hormones. J Reprod Fertil 88:213–221

Takahashi H, Okai Y, Paxton RJ, Hefta LJF, Shively JE (1993) Differential regulation of CEA and BGP by γ-IFN. Cancer Res 53:1612–1619

Tan K, Zelus BD, Meijers R, Liu JH, Bergelson JM, Duke N, Zhang R, Joachimiak A, Holmes KV, Wang JH (2002) Crystal structure of murine sCEACAM1a[1,4]: a coronavirus receptor in the CEA family. EMBO J 21:2076–2086

Tanaka K, Hinoda Y, Takahashi H, Sakamoto H, Nakajima Y, Imai K (1997) Decreased expression of biliary glycoprotein in hepatocellular carcinomas. Int J Cancer 74:15–19

Teglund S, Olsen A, Khan WN, Frängsmyr L, Hammarström S (1994) The pregnancy-specific glycoprotein (PSG) gene cluster on human chromosome 19: fine structure of the 11 PSG genes and identification of 6 new genes forming a third subgroup within the carcinoembryonic antigen (CEA) family. Genomics 23:669–684

Teixeira AM, Fawcett J, Simmons DL, Watt SM (1994) The N-domain of the biliary glycoprotein adhesion molecule mediates homotypic binding: domain interactions and epitope analysis of BGPc. Blood 84:211–219

Thies A, Moll I, Berger J, Wagener C, Brümmer J, Schulze HJ, Brunner G, Schumacher U (2002) CEACAM1 expression in cutaneous malignant melanoma predicts the development of metastatic disease. J Clin Oncol 20:2530–2536

Thompson J, Zimmermann W, Nollau P, Neumaier M, Weber-Arden J, Schrewe H, Craig I, Willocks T (1994) CGM2, a member of the carcinoembryonic antigen gene family is down-regulated in colorectal carcinoma. J Biol Chem 269:32924–32931

Thompson J, Seitz M, Chastre E, Ditter M, Aldrian C, Gespach C, Zimmermann W (1997) Down-regulation of carcinoembryonic antigen family member 2 expression is an early event in colorectal tumorigenesis. Cancer Res 57:1776–1784

Thompson JA, Grunert F, Zimmermann W (1991) Carcinoembryonic antigen gene family: molecular biology and clinical perspectives. J Clin Lab Anal 5:344–366

Thompson JA, Mossinger S, Reichardt V, Engels U, Beauchemin N, Kommoss F, von Kleist S, Zimmermann W (1993) A polymerase-chain-reaction assay for the specific identification of transcripts encoded by individual carcinoembryonic antigen (CEA)-gene-family members. Int J Cancer 55:311–319

Tsutsumi Y, Onoda N, Misawa M, Kuroki M, Matsuoka Y (1990) Immunohistochemical demonstration of nonspecific cross-reacting antigen in normal and neoplastic human tissues using a monoclonal antibody. Comparison with carcinoembryonic antigen localization. Acta Pathol Jpn 40:85–97

Turbide C, Kunath T, Daniels E, Beauchemin N (1997) Optimal ratios of biliary glycoprotein isoforms required for inhibition of colonic tumor cell growth. Cancer Res 57:2781–2788

Varner JA, Brooks PC, Cheresh DA (1995) REVIEW: the integrin alpha V beta 3: angiogenesis and apoptosis. Cell Adhes Commun 3:367–374

Virji M, Watt SM, Barker S, Makepeace K, Doyonass R (1996) The N-domain of the human CD66a adhesion molecule is a target for Opa proteins of Neisseria meningitidis and Neisseria gonorrhoeae. Mol Microbiol 22:929–939

Virji M, Evans D, Hadfield A, Grunert F, Teixeira AM, Watt SM (1999) Critical determinants of host receptor targeting by Neisseria meningitidis and Neisseria gonorrhoeae: identification of Opa adhesiotopes on the N-domain of CD66 molecules. Mol Microbiol 34:538–551

Virji M, Evans D, Griffith J, Hill D, Serino L, Hadfield A, Watt SM (2000) Carcinoembryonic antigens are targeted by diverse strains of typable and non-typable Haemophilus influenzae. Mol Microbiol 36:784–795

Volpert O, Luo W, Liu TJ, Estrera VT, Logothetis C, Lin SH (2002) Inhibition of prostate tumor angiogenesis by the tumor suppressor CEACAM1. J Biol Chem 16:16

von Kleist S, Chavanel G, Burtin P (1972) Identification of an antigen from normal human tissue that crossreacts with the carcinoembryonic antigen. Proc Natl Acad Sci U S A 69:2492–2494

von Kleist S, Winkler J, Migule I, Bohm N (1986) Carcinoembryonic antigen (CEA) expression in early embryogenesis: a study of the first trimester of gestation. Anticancer Res 6:1265–1272

Wagener C, Hain F, Fodisch HJ, Breuer H (1983) Localization of carcinoembryonic antigen in embryonic and fetal human tissues. Histochemistry 78:1–9

Wang J, Springer TA (1998) Structural specializations of immunoglobulin superfamily members for adhesion to integrins and viruses. Immunol Rev 163:197–215

Wang L, Lin SH, Wu WG, Kemp BL, Walsh GL, Hong WK, Mao L (2000) C-CAM1, a candidate tumor suppressor gene, is abnormally expressed in primary lung cancers. Clin Cancer Res 6:2988–2993

Watt SM, Sala-Newby G, Hoang T, Gilmore DJ, Grunert F, Nagel G, Murdoch SJ, Tchilian E, Lennox ES, Waldmann H (1991) CD66 identifies a neutrophil-specific epitope within the hematopoietic system that is expressed by members of the carcinoembryonic antigen family of adhesion molecules. Blood 78:63–74

Watt SM, Teixeira AM, Zhou GQ, Doyonnas R, Zhang Y, Grunert F, Blumberg RS, Kuroki M, Skubitz KM, Bates PA (2001) Homophilic adhesion of human CEACAM1 involves N-terminal domain interactions: structural analysis of the binding site. Blood 98:1469–1479

Wikström K, Kjellström G, Öbrink B (1996) Homophilic intercellular adhesion mediated by C-CAM is due to a domain 1-domain 1 reciprocal binding. Exp Cell Res 227:360–366

Williams AF, Barclay AN (1988) The immunoglobulin superfamily—domains for cell surface recognition. Annu Rev Immunol 6:381–405

Williams RK, Jiang GS, Snyder SW, Frana MF, Holmes KV (1990) Purification of the 110-kilodalton glycoprotein receptor for mouse hepatitis virus (MHV)-A59 from mouse liver and identification of a nonfunctional, homologous protein in MHV-resistant SJL/J mice. J Virol 64:3817–3823

Williams RK, Jiang GS, Holmes KV (1991) Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins. Proc Natl Acad Sci U S A 88:5533–5536

Zhou GQ, Baranov V, Zimmermann W, Grunert F, Erhard B, Mincheva-Nilsson L, Hammarström S, Thompson J (1997) Highly specific monoclonal antibody demonstrates that pregnancy-specific glycoprotein (PSG) is limited to syncytiotrophoblast in human early and term placenta. Placenta 18:491–501

Zimmer R, Thomas P (2001) Mutations in the carcinoembryonic antigen gene in colorectal cancer patients: implications on liver metastasis. Cancer Res 61:2822–2826

Zimmermann W (2002) Carcinoembryonic antigen. In: Creighton T (ed) Wiley Encyclopedia of Molecular Medicine. John Wiley and Sons, New York, pp 459–462

Zimmermann W, Ortlieb B, Friedrich R, von Kleist S (1987) Isolation and characterization of cDNA clones encoding the human carcinoembryonic antigen reveal a highly conserved repeating structure. Proc Natl Acad Sci U S A 84:2960–2964