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GUEST COMMENTARY

Four Viruses, Two Bacteria, and One Receptor: Membrane Cofactor Protein (CD46) as Pathogens' Magnet

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CD46 (also known as membrane cofactor protein or MCP) is a regulator of complement activation that is expressed in most or all human nucleated cell types. CD46 was initially identified as binding and inactivating C3b and C4b complement products, a process protecting human cells from lysis by autologous complement (18). It also serves as a receptor for several human pathogens: an enveloped RNA virus (measles virus [MV]), an enveloped DNA virus (human herpesvirus 6), a nonenveloped DNA virus (adenovirus of different serotypes), and two types of bacteria (*Streptococcus pyogenes* and pathogenic *Neisseria*) (13, 27). Moreover, bovine CD46 was recently shown to serve as a receptor for bovine viral diarrhea virus (BVDV), an enveloped RNA virus. That six different pathogens selected the same molecule as a port of cell entry is of interest not only for microbiologists and immunologists, who wonder what makes CD46 such a seductive molecule, but also for gene therapists, who are seeking opportunities for targeting disease sites.

BOVINE CD46 IS A CELLULAR RECEPTOR FOR A PESTIVIRUS

A recent study by Maurer et al. (22) identified bovine CD46 as the receptor for BVDV, a small, enveloped RNA virus that belongs to the genus *Pestivirus* within the family *Flaviviridae*. BVDV is an important pathogen of cattle and accounts for syndromes of the intestinal, respiratory, and reproductive tracts. The search for the BVDV receptor started with a monoclonal antibody that blocked infection. This antibody was shown to recognize a bovine protein with a significant sequence homology to porcine and human CD46 (22). Expression of bovine CD46 in porcine cells increased susceptibility of these cells to infection with one strain of BVDV, whereas expression of bovine CD46 in human cells enhanced virus binding but did not allow productive infection. Thus, BVDV tropism must be influenced by factors other than bovine CD46 availability: alternative receptors, coreceptors, or postentry events.

The identification of a ubiquitous molecule serving as the BVDV receptor also raised questions regarding its relevance for tropism and pathogenesis. BVDV replicates in essentially all organs and tissues, but initially this virus selectively infects the tonsils and then spreads to other lymphatic tissues before the occurrence of viremia (17). Even if CD46 is an ubiquitous protein, it was recently shown that certain interactions of this protein occur specifically in immune cells. In particular, coengagement of CD46 and CD3 in the presence of interleukin 2 induces the development of cells with a T-regulatory 1 phenotype (14). This observation raises the possibility that BVDV and other pathogens interfere with fundamental processes in the immune response by altering CD46 function (see last section). Thus, BVDV interactions with CD46 of immune cells may induce fundamentally different effects as with other cell types.

MV AND CD46: ATTENUATION THROUGH STRONGER BINDING?

There are interesting parallels between BVDV and MV: both pathogens initially spread in lymphatic cells and induce a strong immunosuppression. MV was the first pathogen shown to use CD46 as a receptor: the glycoproteins of the attenuated strain Edmonston were used to set up a screening system based on the inhibition of cell fusion. A library of 3,000 monoclonal antibodies raised against surface proteins expressed in MV-permissive cells yielded one clone whose supernatant interfered with MV glycoprotein-induced fusion (26). The protein recognized by this monoclonal antibody was characterized as being CD46 and was shown to support MV entry in rodent cells (7, 25). MV did replicate in rodent cells expressing human CD46 but not at the same levels reached in human cells.

The question of the relevance of an ubiquitous receptor for the pathogenesis of a lymphotropic virus was raised again when it was shown that the H protein of the attenuated Edmonston strain interacted more efficiently with CD46 than the proteins of certain wild-type MV strains (16). When it was then found that wild-type strains rely on SLAM (signaling lymphocyte activation molecule or CD150) for cell entry (34), the receptor-tropism question seemed solved to the advantage of SLAM: this molecule is expressed only in certain immune cells, a fact that better explains the immunosuppressive nature of MV infection. In addition, two other immunosuppressive MV relatives, the morbilliviruses canine distemper virus and rinderpest virus, use canine and bovine SLAM but not CD46 as their receptors, respectively (9, 35).

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Nevertheless, it remains conceivable that MV interactions with CD46 contribute to tropism and immunosuppression. Certain wild-type MVs interact with CD46 (19, 31); the binding affinity of the respective attachment proteins and CD46 has not been determined, but it is lower than that of the attachment protein of the vaccine strain (120 nM) (4, 29). A scenario for MV attenuation considers that it is related to early efficient entry of the vaccine strain in nonimmune cells, dampening the effects of the timely attack of SLAM-expressing lymphocytes and macrophages strategically executed by wild-type strains.

CD46 MEDIATES ATTACHMENT AND ENTRY OF DIFFERENT ADENOVIRUSES

Adenoviruses are icosahedral, nonenveloped DNA viruses. Most of the 51 adenovirus serotypes are only mildly pathogenic; they are grouped in six species, A to F. Members of all species except B use the coxsackievirus-adenovirus receptor (CAR), as a port of cell entry (2, 28). Entry of a group D serotype (Ad37) appeared to be an exception because it requires sialic acid rather than the CAR protein (1). Four recent publications have identified human CD46 as a receptor for different adenovirus serotypes: Ad3 (33), Ad11 (28), Ad35 (8), and Ad37 (38). These data collectively indicate that CD46 is a receptor for several group B adenoviruses, including Ad3, Ad11, and Ad35, and for a group D adenovirus, Ad37.

The data presented are in each case compelling because receptor identification and characterization were based on different techniques. Initially Segerman et al. (32), based on gifted insight, postulated that CD46 is a cellular receptor for Ad11 and then showed this by rendering rodent cells permissive to Ad11 infection through CD46 expression and by blocking binding and infection with soluble fiber knob (the viral attachment protein) or anti-CD46 antibodies. Independently, Gaggar et al. (8) produced a recombinant Ad35 fiber knob protein and used it to purify potential receptors. The cellular proteins binding to the recombinant knob protein were analyzed by mass spectroscopy, and CD46 was identified. Gaggar et al. then went on to show that CD46 supports cell entry and productive Ad35 replication and that it also supports the attachment of the other species B adenoviruses, serotypes 11, 14, 16, and 50. In another independent approach, Wu et al. (38) characterized the receptor for Ad37 (group D), a serotype associated with epidemic keratoconjunctivitis. CD46 again was identified with other proteins as a candidate for binding to Ad37 in a virus overlay assay. It was then shown that nonpermissive cells promoted adenovirus replication upon CD46 expression and that an antibody specific for the most external domain of CD46 blocked Ad37 infection of two human cell lines. In a fourth study Sirena et al. (33) screened an expression library made from cDNAs of cells that efficiently bound Ad3. The rodent cells that gained the capacity of binding fluorescent Ad3 particles expressed either one of the major CD46 isoforms. These cells internalized Ad3 and developed cytopathic effects. These authors also showed that Ad3 binds with an affinity of 0.3 nM human cells and CD46-expressing rodent cells but not control rodent cells. Finally it was shown that the CD46 ectodomain binds the Ad3 fiber head.

RECEPTOR TISSUE DISTRIBUTION AND VECTOR TARGETING

The above data prove beyond reasonable doubt that human CD46 is a receptor for several adenoviruses. Again the issue of the relevance of CD46 for viral tropism has to be raised, and again the existence of alternative receptors, coreceptors, and postentry virulence determinants has to be invoked to explain the distinctive tropism and pathology of the different adenovirus-based serotypes. An interesting observation in this respect is that the CD46 C isoform, which is highly expressed in the eye (cornea/conjunctiva) and brain, is preferentially used by Ad37.

In the perspective of adenovirus vector applications, the fact that certain strains contact a ubiquitous protein rather than the much less readily available CAR is good news. Present gene therapy protocols rely on vectors derived from Ad2 and Ad5, two species C adenoviruses that use CAR as a receptor. However, CAR is expressed only in a few tissues, and efficient adenovirus-mediated transgene expression is associated with CAR availability (11). The question now becomes how successful gene transfer protocols can be, based on CD46 usage and, more in general, whether and how a ubiquitous molecule can be exploited for vector targeting.

The fact that certain viruses become attenuated when they bind tightly to ubiquitous receptors expressed at high levels (reference 20 and references therein) is a warning of the difficulties that have to be overcome. For example, tick-borne encephalitis virus adapts to negatively charged heparan sulfate: local patches of predominantly positively surface charges evolve in its attachment protein (20). The positively charged amino acids are selected in tissue culture, but when a tissue culture-adapted virus is passed into mice neuroinvasiveness is attenuated.

This does not mean that any level of heparan sulfate binding may preclude vector targeting. In contrast, low-affinity binding may concentrate viral particles in a given organ expressing high heparan sulfate levels and facilitate subsequent interactions with a high-affinity receptor or a coreceptor. Detailed knowledge of the organ distribution and concentration of receptor and coreceptor molecules is necessary to predict the efficiency of targeting approaches. Characterization of the interactions of the viral attachment proteins with their receptors is also required to plan the production of recombinant viruses with carefully balanced affinities to different receptors. Towards this it was shown that selectively receptor-blind viruses can be created through the introduction of point mutations in their attachment proteins (5, 36, 39).

The art of vector targeting is only beginning to develop into a science (6, 37). Ligand-directed targeting of viral vectors to disease sites is based on the present, incomplete framework of knowledge about receptor hierarchy and tissue distribution and proceeds slowly. Alternative approaches to targeting that rely on the characterization of the tropism of viruses collected from primates or other mammals are being pursued (10). Moreover, chimeras of different viral species are being produced in the perspective of generating vector libraries with novel receptor specificities from which individual vectors can be selected. These approaches will synergize with studies of viral structure and assembly to guide the development of vectors with incrementally higher tissue and cell specificity.

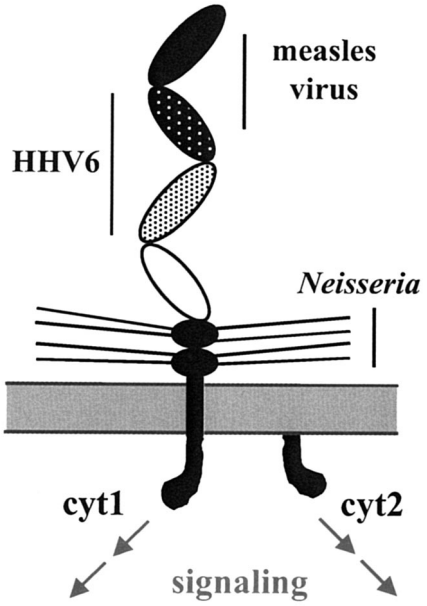

FIG. 1. CD46 structure, domains interacting with different pathogens, and postulated cytoplasmic tail-dependent signaling are shown. Large elongated ovals, CCP modules; small ovals, STP domains; thin lines originating in the STP domains, O-linked oligosaccharides; cyt1 and cyt2, alternative cytoplasmic tails; shaded bar, plasma membrane; and arrows, postulated signaling cascades.

WHY CD46?

Is it a particular structure or structural pattern, a strategic location in a subcompartment of the cell surface, or another characteristic that predestines CD46 to be a port of entry for so many pathogens? CD46 is a type I membrane glycoprotein (Fig. 1). From its amino terminus there are four tandem complement control protein (CCP) modules followed by one or two heavily O-glycosylated serine/threonine/proline-rich (STP) domains, a transmembrane region, and two alternative cytoplasmic tails (15). As shown in Fig. 1, pathogens recognize different structures in the CD46 ectodomain: MV binds to the two external CCP modules, human herpesvirus 6 binds to modules 2 and 3, and pathogenic *Neisseria* relies on the STP domains for cell attachment (3, 12, 24, 30). Preliminary data derived only from antibody competition experiments suggest that different adenovirus strains may contact different CCP modules (32, 38). Taken together, these data indicate that there is not a single structural target for pathogens on CD46.

The cellular mechanisms facilitating cell entry of the different CD46-dependent pathogens are also fundamentally different: no subcellular compartment is consistently implicated in the pathogens' interactions. MV and human herpesvirus 6 enter cells by pH-independent fusion of their membrane with the plasmalemma, adenovirus species C enters via clathrin-dependent endocytosis (23), and other adenovirus species are likely to use similar endocytotic mechanisms; *Neisseria* does not enter cells routinely but relies on CD46 for the initial attachment, facilitating subsequent adhesion. Table 1 shows different pathogens' interactions with CD46.

Thus, the question of what makes CD46 a pathogen's magnet remains, and the answer may have to be sought inside the cell. If different pathogens use the same CD46 function to create a favorable environment and elude detection and destruction, how do they elicit a signal? Probably through the CD46 intracellular domain. CD46 has two alternative cytoplasmic tails that result from alternative splicing. Since these tails are only 23 and 16 residues in length, it was initially assumed that their contribution to the conditioning of the cytoplasm may not be significant. However, two recent studies have shown that CD46 can drive T-cell differentiation (14) and that the CD46 cytoplasmic tails have a divergent role in T-cell-induced inflammation (21). Thus, CD46 not only regulates complement activation but fine-tunes the T-cell-mediated cellular response, thereby bridging innate and acquired immunity. It is conceivable that all the CD46-targeting pathogens interfere with cytoplasmic tail-elicited intracellular signaling pathways, thereby unbalancing the immune response. Once again, pathogens are trying to teach us cell biology and immunology. Microbiologists and gene therapists should pay attention.

TABLE 1. Pathogen interactions with CD46

Pathogen	Attachment protein	Type of interaction
MV	Hemagglutinin	Binding leads to membrane fusion
Human herpesvirus 6	Glycoprotein H	Binding leads to membrane fusion
Adenovirus (different serotypes)	Knob	Binding results in endocytosis
BVDV	E2[a]	Probably endocytosis
Neisseria gonorrhoeae and *Neisseria meningitides*	Pili	Initial attachment, facilitating adhesion
S. pyogenes	Unknown	Unknown

[a] Till Ruemenapf, personal communication.

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