PERSPECTIVES: MICROBIOLOGY

A Welcome Mat for Leprosy and Lassa Fever

Patricia G. Spear

Two of the most feared human diseases are leprosy and Lassa fever. Although these infectious diseases differ markedly in etiology and pathogenesis, the causative agent of each disease binds to target cells through interactions with a common receptor, α-dystroglycan. In this issue on page 2079, Cao *et al.* (*1*) demonstrate that several members of the arenavirus family, including Lassa fever virus, bind directly to α-dystroglycan and that expression of this peripheral membrane protein confers on cells susceptibility to viral infection. On page 2076, Rambukkana *et al.* (*2*) show that *Mycobacterium leprae*, the bacterium responsible for leprosy, or Hansen's disease, can bind to α-dystroglycan via an intermediary, laminin-2, and that bacteria coated with a fragment of laminin-2 bind to cultured Schwann cells, causing aggregation of cell surface–associated α-dystroglycan.

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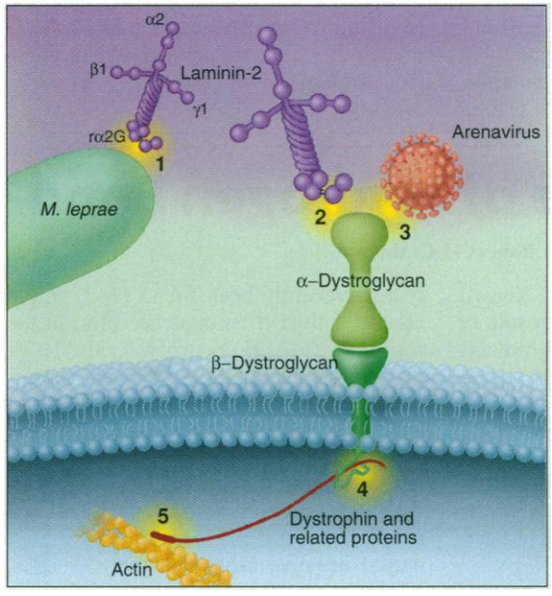
A single gene encodes α-dystroglycan and β-dystroglycan, which are derived from a precursor polypeptide by posttranslational cleavage (*3*). β-Dystroglycan is an integral membrane protein, whereas α-dystroglycan is membrane-associated through its noncovalent interaction with the extracellular domain of β-dystroglycan. These proteins are highly conserved in the animal kingdom and are expressed in a variety of cell types from early development into adulthood. Emerging evidence indicates that α- and β-dystroglycan provide important physical linkages between components of basement membranes and cytoplasmic proteins that bind to the actin cytoskeleton.

For example, in skeletal and cardiac muscle, structural integrity of the sarcolemma is thought to depend in part on binding of the cytoplasmic protein, dystrophin, to both actin and the cytoplasmic tail of β-dystroglycan and binding of α-dystroglycan to laminin-2 in the basal lamina. Laminins (*4*) are composed of three polypeptide chains designated α, β, and γ. The multiple isoforms of laminin differ in their constituent chains. Laminin-2 is composed of α2, β1, and γ1. Human mutations affecting dystrophin, dystrophin-associated proteins, or laminin-2 are the causes of various muscular dystrophies or cardiomyopathies (*5*). Homozygous deletion of the gene encoding dystroglycan is lethal at the embryonic stage in mice (*6*) and would presumably also be lethal in humans.



Point of entry. Interactions of α-dystroglycan with *Mycobacterium leprae* and arenaviruses such as Lassa fever virus. Arenaviruses bind directly to α-dystroglycan (**3**) and this binding can lead to viral entry. *M. leprae* binds to laminin-2, specifically to the G domain of the α2 chain (**1**), and laminin-2 binds to α-dystroglycan (**2**). The normal physiological role of α-dystroglycan and β-dystroglycan is to link components of the extracellular matrix to the actin cytoskeleton. Laminins bind to α-dystroglycan (**2**). Dystrophin and related proteins bind to the cytoplasmic domain of β-dystroglycan (**4**) and to actin (**5**).

Dystroglycan is present and functional in cell types other than muscle, such as epithelial cells and cells of the nervous system. Interactions of α-dystroglycan with laminins and of β-dystroglycan with dystrophin or dystrophin-related proteins are a common theme, suggesting the importance of this transmembrane complex in physical linkage, and perhaps signaling, between the extracellular matrix and cytoskeletal components. The binding partners of dystroglycan in different cell types and in specialized regions of a single cell type may differ. For example, at neuromuscular junctions, α-dystroglycan is found in association with agrin, which has a laminin-like domain, and β-dystroglycan is associated with utrophin, which is related to dystrophin.

In the study by Cao *et al.* (*1*), two arenaviruses, lymphocytic choriomeningitis virus (LCMV) and Lassa fever virus, were found to bind to a protein on blots of cell membrane proteins that had been fractionated by SDS-PAGE. Partial amino acid sequencing of the isolated protein suggested that it was α-dystroglycan. A variety of arenaviruses were then tested for their ability to bind to purified α-dystroglycan, and all but one could bind. Evidence that dystroglycan can serve as a receptor for viral entry came from demonstration that mutant mouse embryonic stem cells in which both copies of the dystroglycan gene were knocked out were resistant to LCMV infection, whereas the parental cells were susceptible, as were the knockout cells infected with an adenovirus vector expressing dystroglycan. Previous studies have shown that the LCMV glycoprotein GP-1 mediates the binding of virus to cells, and presumably it is the viral ligand for α-dystroglycan.

The natural hosts for arenaviruses (*7*) that infect humans are usually rodents, in which infections are benign. Transmission of virus to humans, principally via the respiratory route, can result in severe hemorrhagic or neurologic disease. Studies of tissues from animals infected with LCMV and other arenaviruses, and of a limited number of human specimens, reveal that the virus can infect a wide range of cell types, consistent with the distribution of α-dystroglycan. Infected cells exhibit relatively little direct virus-induced cytopathology. Immune responses are provoked with potentially devastating effects, however. Hemorrhage is probably not due to direct effects of virus on endothelial cells, but to inflammatory responses. In fact, endothelial cells may not be susceptible to viral entry. It remains to be determined whether cells that can be infected by arenaviruses in vivo necessarily express α-dystroglycan or whether alternative receptors can also be used for viral entry. Presumably alternative receptors are used by

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the arenavirus Guanarito (and perhaps others not tested) because Guanarito did not bind to isolated α-dystroglycan.

The neuropathy of leprosy (*8*) is caused in part by invasion of peripheral nerves by *M. leprae*. In lepromatous leprosy, protective immune responses are absent and bacteria can spread freely through skin and into peripheral nerves. Spread to deeper tissues is limited because the bacteria reproduce best at temperatures of 27° to 30°C. In tuberculoid leprosy, protective immune responses are present and the disease is more circumscribed, although damage to nerves still results. The Schwann cell is an important target for bacterial invasion. In the endoneurium of peripheral nerve, Schwann cells are covered by basal lamina, composed of laminin, type IV collagen, entactin/nidogen, and heparan sulfate proteoglycans. In a previous study (*9*), Rambukkana and colleagues demonstrated that *M. leprae* could bind to the G domain of the α2 chain of laminin-2 and that a recombinant protein composed of this G domain could mediate the binding of *M. leprae* to various cell types, including Schwann cells.

In the current study (*2*), Rambukkana *et al.* have shown that α-dystroglycan is a cell receptor for the binding of G domain–coated bacteria to Schwann cells and that this binding results in aggregation of cell surface α-dystroglycan and colocalization of bacteria with these aggregates. Moreover, soluble α-dystroglycan can partially inhibit the binding of coated bacteria to the cells. Characterization of the interaction between bacteria coated with the α2 G domain and isolated α-dystroglycan showed that bacterial adherence to immobilized α-dystroglycan was inhibited by EDTA (but not by heparin) and by treatments that would alter the carbohydrate chains on α-dystroglycan. Possibly the G domain has a lectin-like activity separable from its heparin-binding activity. It is not known how *M. leprae* binds to laminin-2. We can look forward to future studies designed to define the molecular details of interactions between these viral and bacterial agents of disease and dystroglycan, the pathological consequences of these interactions, and the therapeutic potential of blocking them.

References

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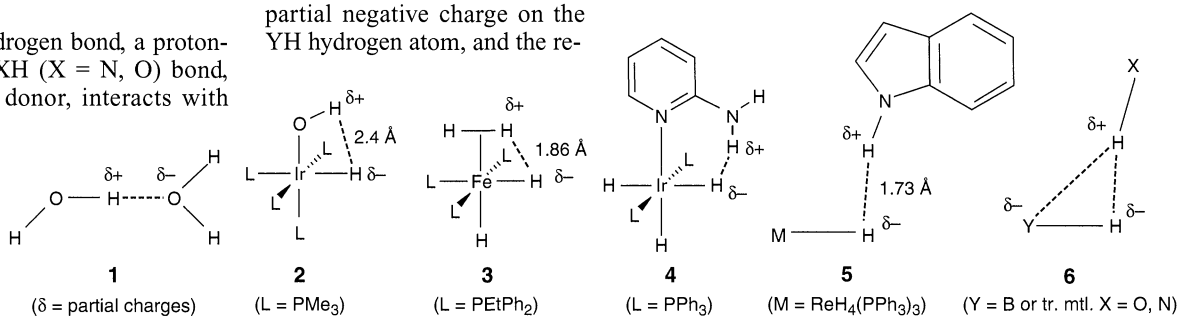
PERSPECTIVES: CHEMISTRY

A New Type of Hydrogen Bond

Robert H. Crabtree

The classical hydrogen bond, known for over 50 years, plays a key role in the structure and function of biological molecules. Hydrogen bonds are responsible for the strength of materials, such as wood or a spider's web, and molecular binding, such as base pairing in DNA. Recently, however, a whole new world of hydrogen bonding has come to light, in which metals and hydrides can form unusual hydrogen bonds.

In the typical hydrogen bond, a protonic hydrogen of an XH (X = N, O) bond, the hydrogen bond donor, interacts with the basic lone pair of an electronegative atom, the hydrogen bond acceptor, as in the gas phase water dimer (see structure **1**). In the last 15 years or so, it has become clear that the π electrons of an aromatic ring can sometimes act as an H bond acceptor to form an X–H...π hydrogen bond, but this interaction tends to be weaker because π-bonding electrons are less basic than lone pairs. By extrapolation, one would expect a σ bond to be an even less effective H bond acceptor. A striking reverse of this expectation has only very recently been found (*1–3*) in the case of a Y–H σ bond as acceptor. In the resulting X–H...H–Y structure, the H...H distance, typically 1.8 Å, is much shorter than the normal H...H contact of 2.4 Å. This attractive interaction between two hydrogens has come to be called the "dihydrogen bond." Y is always an electropositive atom such as boron or a transition metal, however. This leads to a partial negative charge on the YH hydrogen atom, and the resulting H...H interaction can, therefore, be thought of as an attractive proton-hydride interaction.



1 (δ = partial charges) **2** (L = PMe_3) **3** (L = $PEtPh_2$) **4** (L = PPh_3) **5** (M = $ReH_4(PPh_3)_3$) **6** (Y = B or tr. mtl. X = O, N)

Compounds **2** and **3** were both found by neutron diffraction in 1990 to have close H...H distances between oppositely charged hydrogen atoms (*1*). Since 1994, our group at Yale (*2*), Bob Morris' group at the University of Toronto (*3*), and many others have found that such bonding is general, as in compound **4**. X–H...H–Y structures were not found earlier probably because they tend to be unstable and readily lose H_2 to give a species with a new X–Y bond. Indeed, dihydrogen-bonded species are probably transient intermediates whenever a hydride, YH, is protonated by an acid, XH:

$$X\text{–}H + H\text{–}Y \rightarrow X\text{–}H...H\text{–}Y \rightarrow X\text{–}Y + H_2$$

Dihydrogen bonding need not be intramolecular, as in structures **2** to **4**, because the intermolecular case **5** also has a very short H...H distance (1.73 Å) (*2*). Various techniques suggest that the bond strength is generally about 4 to 6 kcal/mol, much the same as in a classical hydrogen bond of average strength (*2*).

The recent discovery (*4*) of N–H....M hydrogen-bonded structures shows that metal atoms can themselves act as H bond acceptors and suggests that there may be a direct N–H...M component to the N–H...H–M interaction. This helps explain the characteristic bent shape (see **6**) usually found for the X–H...H–Y structure but raises the question of whether X–H...H–Y structures should be considered as pre-

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