PERSPECTIVES: MICROBIOLOGY

Deconstructing Vancomycin

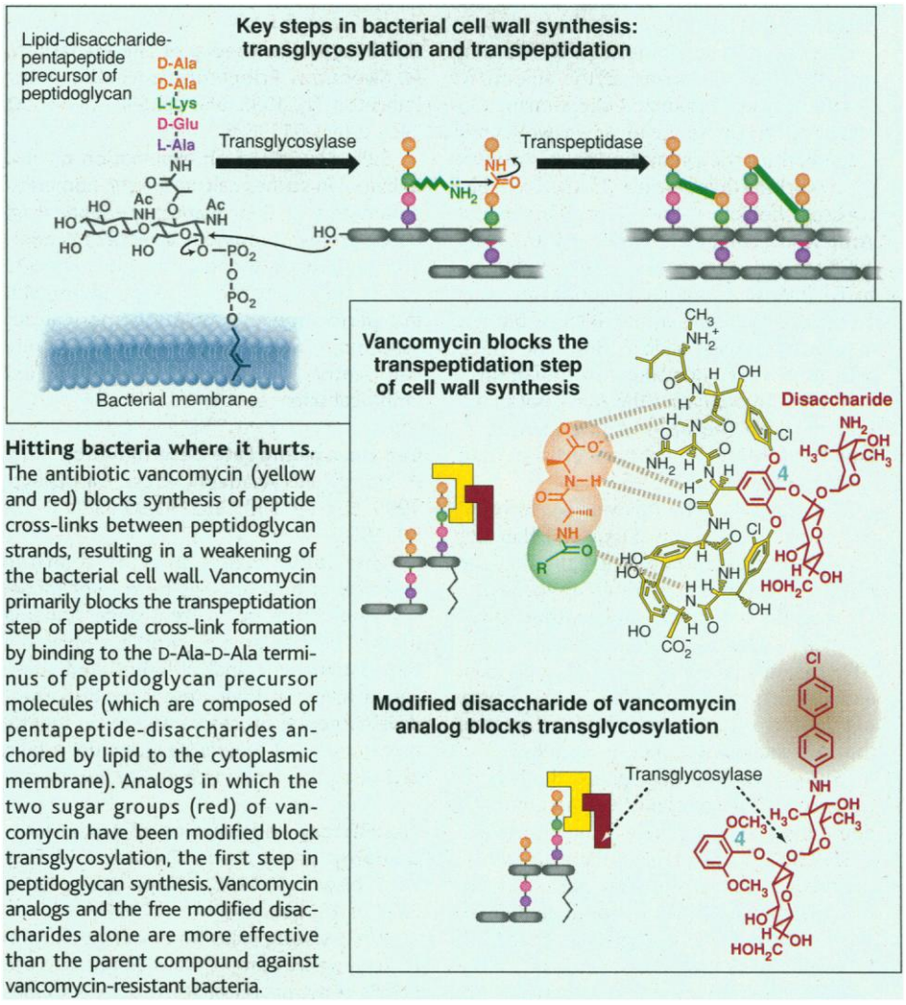
Christopher Walsh

Vancomycin is an antibiotic that occupies an important niche in the treatment of life-threatening infections caused by Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis*. This glycopeptide is crucial for treating infections in, for example, cancer patients undergoing chemotherapy and renal patients on dialysis. The widespread prevalence of methicillin-resistant *S. aureus* has made vancomycin the antibiotic of last resort for eliminating multi–drug-resistant Gram-positive bacteria. But recent reports of patients infected with methicillin-resistant *S. aureus* that also proved to be resistant to vancomycin (*1*, *2*) raise the specter of the worst kind of antibiotic-resistant superbug (*3*). The arrival of vancomycin-resistant *S. aureus* follows the emergence of vancomycin-resistant enterococcus, which has plagued hospitals for the last 10 years.

But all may not be lost in the fight against multi–drug-resistant superbugs, at least not according to the report by Ge and colleagues on page 508 of this issue (*4*). By modifying the sugar groups attached to vancomycin's peptide backbone, these investigators synthesized analogs that were not only more efficient than the parent compound at dispatching vancomycin-resistant bacteria but were also better at killing vancomycin-sensitive organisms. The authors showed that the vancomycin analogs blocked an earlier step (transglycosylation) in bacterial cell wall synthesis than did vancomycin, and that the modified sugar groups themselves had substantial antibacterial activity. These results should redirect attention to designing new, sugar-based antibiotics that inhibit the transglycosylase step in bacterial cell wall synthesis.

Vancomycin interdicts bacterial growth primarily by blocking the cross-linking of adjacent peptidoglycan strands by peptide bonds during synthesis of the bacterial cell wall (see the figure, top). Without sufficient cross-linking, the cell wall becomes mechanically fragile and the bacteria lyse when subjected to changes in osmotic pressure. Vancomycin binds to the D-alanine-D-alanine (D-Ala-D-Ala) terminus of the pentapeptide portion of the peptidoglycan precursor before cross-linking. The D-Ala-D-Ala dipeptide forms complementary hydrogen bonds with the peptide backbone of vancomycin. It is thought that the vancomycin-peptidoglycan complex physically occludes the subsequent action of transpeptidase enzymes (the targets of penicillins) and in so doing blocks formation of the peptide cross-bridges that confer strength on the peptidoglycan (see the figure, bottom). As a secondary event, the transglycosylation step that connects the disaccharide unit of the pentapeptide precursor to existing glycan strands also appears to be inhibited by vancomycin, although to a lesser extent. The two most prevalent clinical isolates of vancomycin-resistant enterococci, Van A and Van B, recans of resistant bacteria. However, modifying the disaccharide sugar of vancomycin rather than the peptide backbone proved to be a novel approach to making effective analogs. The synthesis of the vancomycin peptide backbone was recently accomplished by the research groups of Evans (*7*) and Nicolaou (*8*). Kahne's team then succeeded in attaching the disaccharide vancosamine-glucose to the peptide backbone, thus completing the synthesis of the vancomycin molecule (*9*). It proved program the peptidoglycan synthetic machinery, replacing the D-Ala-D-Ala dipeptide with D-Ala-D-lactate. The loss of a crucial hydrogen bond between vancomycin and the terminal dipeptide results in a decrease (by three orders of magnitude) in the binding affinity of the antibiotic for the peptidoglycan (*5*, *6*).

Much interest surrounds methods to modify vancomycin to combat resistance, but the heptapeptide backbone of vancomycin has a rigid cup-shaped architecture that prevents it from binding to the D-Ala-D-lactate dipeptide in the peptidogly-



Hitting bacteria where it hurts. The antibiotic vancomycin (yellow and red) blocks synthesis of peptide cross-links between peptidoglycan strands, resulting in a weakening of the bacterial cell wall. Vancomycin primarily blocks the transpeptidation step of peptide cross-link formation by binding to the D-Ala-D-Ala terminus of peptidoglycan precursor molecules (which are composed of pentapeptide-disaccharides anchored by lipid to the cytoplasmic membrane). Analogs in which the two sugar groups (red) of vancomycin have been modified block transglycosylation, the first step in peptidoglycan synthesis. Vancomycin analogs and the free modified disaccharides alone are more effective than the parent compound against vancomycin-resistant bacteria.

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possible to modify the free amino group in the vancosamine sugar moiety with hydrophobic substituents, such as biphenyls, and to show that this modification increased activity against vancomycin-resistant enterococci (*10*). Now Ge *et al.* have gone further, using their expertise in carbohydrate chemistry to obtain unanticipated findings about vancomycin analogs.

Their first intriguing discovery was the retention of substantial antibacterial activity in a chlorobiphenyl vancomycin derivative that was missing the first (leucyl) residue. This meant that recognition of the D-Ala-D-Ala terminus by the analog was abrogated, ruling out the conventional high-affinity interaction between the antibiotic and the peptidoglycan as the mode of drug action. The investigators further pruned the vancomycin skeleton down to the modified vancosamine-glucose disaccharide and found, to their amazement, that the disaccharide alone retained powerful antibiotic activity. They used permeabilized *Escherichia coli* bacteria to determine which step in the peptidoglycan synthesis pathway was blocked by the disaccharide. In contrast to vancomycin, which primarily abrogates transpeptidation, the vancomycin analog and its disaccharide fragment alone selectively blocked the transglycosylation step of peptidoglycan synthesis.

It is thought that vancomycin binds to D-Ala-D-Ala termini in the non–cross-linked mature peptidoglycan, and also in the lipid-disaccharide pentapeptide precursor molecules that are substrates for incorporation into expanding peptidoglycan chains. It now appears that the vancomycin analog and its disaccharide fragment directly interact with one or more of the transglycosylases involved in oligomerization of the glycan strands. The discovery that these enzymes are targets for the modified sugars of vancomycin reveals simple strategies for defining which transglycosylases are involved in bacterial cell wall synthesis and for designing a new class of antibiotics by manipulation of vancosamine and glucose rings. To what extent the dimethoxyphenyl ring component (mimicking residue 4 of vancomycin) of the disaccharide fragment is crucial for anchoring the disaccharide in the bacterial cell membrane and for the fragment's antibacterial activity will also need to be explored.

All in all, the deconstructionist approach to vancomycin appears to have struck gold with the discovery of a disaccharide fragment of a vancomycin analog that is more powerful than vancomycin itself. These results may accelerate the discovery and development of simple sugar-based fragments that are equally adept at killing vancomycin-sensitive and vancomycin-resistant pathogenic bacteria by targeting not the transpeptidation step but the transglycosylation step of peptidoglycan synthesis.

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PERSPECTIVES: SIGNAL TRANSDUCTION

Nuclear Fusion of Signaling Pathways

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Extracellular factors that regulate cell growth and differentiation often bind to receptors at the cell surface. Signals are then transduced to the nucleus where they activate specific transcription factors that elicit changes in the pattern of gene transcription. We know of many linear, intracellular signal transduction pathways, and often there is cross talk between them. But how and where they intersect and whether this cross talk results in an enhanced signal (synergy) or a reduced signal (antagonism) is not known. One answer to this conundrum entails the homologous transcriptional coactivator molecules p300 and CREB-binding protein (CBP). These huge nuclear proteins interact with numerous transcription factors through distinct domains (see the figure). They are thought to be bridges that connect individual transcription factors to the basal transcription machinery, thus helping to activate transcription (*1*). However, what remains unclear is whether p300 or CBP interacts simultaneously with more than one transcription factor in a promoter complex. Studies in mice deficient in p300 or CBP show that these molecules are essential and are in limited supply within the cell. Thus, competition for p300 and CBP may explain negative interference between different signaling pathways (*2*).

Now, on page 479 of this issue, Nakashima *et al.* (*3*) report on an alternative way in which signaling pathways can communicate with each other. They show that p300 facilitates synergistic cross talk between two different signaling pathways—activated by the cytokines LIF (leukemia inhibitory factor) and BMP2 (bone morphogenetic protein 2)—which together stimulate the differentiation of fetal neuroepithelial cells into astrocytes. LIF is a survival and differentiation factor for neurons, and a deficiency in LIF or its receptor results in changes in astrocytes in the central nervous system (*4*). Similarly, the BMP2 protein is expressed throughout neural development and promotes the differentiation of neural progenitor cells into astrocytes that have structural, trophic, and immunomodulatory functions in the brain (*5*).

The LIF and BMP2 cytokines induce distinct signal transduction pathways that each activate a different transcription factor (STAT3 and Smad1, respectively) at the cytoplasmic face of the plasma membrane (see the figure). LIF binds to and induces the heterodimerization of transmembrane receptor subunits, resulting in the activation of the JAK family of protein tyrosine kinases. The activated JAKs then phosphorylate the transcription factor STAT3 on a tyrosine residue. Phosphorylated STAT3 dimerizes, translocates to the cell nucleus, binds to DNA, and activates the transcription of target genes (*6*). In the same way, dimers of BMP2 bind to and induce tetramerization of the two types of BMP receptor, which have serine-threonine kinase activity. This results in the phosphorylation of the Smad1 transcription factor, which associates with Smad4, moves to the nucleus, and stimulates expression of target genes (*7*).

As both STAT and Smad family proteins have been shown to bind to p300 (*8*), Nakashima *et al.* investigated the possible interactions between Smad1, STAT3, and p300 (*3*). Upon phosphorylation by BMP receptors, Smad1 interacted with p300; STAT3 also bound to p300 but independently of tyrosine phosphorylation, suggesting that the phosphorylation-induced dimerization that is essential for the binding of STAT3 to DNA is not required for

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