Biomedicine Select

Cell

In conjunction with this special issue on the interactions between pathogens and their hosts, Biomedicine Select showcases new findings about a variety of microbes some of which have a major impact on global health. Multidrug resistance in bacteria, recent advances in influenza research, and the use of siRNAs as potential therapeutics against viruses are discussed.

Dishing the Dirt on Multidrug Resistance

Colonies of sporulating *Streptomyces* bacteria that are multidrug resistant. Photo courtesy of Gerard Wright.

Antibiotic-resistant bacteria are a serious threat to human health. A recent study by D'Costa et al. reveals that multidrug-resistant microbes are far more prevalent than previously imagined. This group examined soil microbes for antibiotic resistance. First, they isolated spore-forming bacteria from different environments and then selected the actinomycetes (confirmed by sequencing 16S ribosomal DNA) for further study. They focused on 480 actinomycetes of the genus *Streptomyces*, because more than half of all antibiotics are derived from this species. Hence, members of this species are likely to encode resistance elements for the antibiotics they produce as a means of self-protection. These 480 strains were individually tested for their ability to grow in the presence of 21 antibiotics including natural (such as erythromycin), semisynthetic (such as cephalexin), and synthetic (ciprofloxacin) antibiotics. Strikingly, every strain was found to be resistant to seven or eight antibiotics, even newer antibiotics. In some cases, resistance was due to the production of inactivating enzymes that metabolized the drug. One such drug was rifampicin, a derivative of a natural product, which is used routinely to treat mycobacterial infections (which cause diseases such as tuberculosis). Forty percent of the bacteria resistant to rifampicin could inactivate the antibiotic. Interestingly, in a clinical setting, resistance to rifampicin is caused by mutations in the bacterial target of this drug, the β subunit of RNA polymerase. Other mechanisms of resistance include glycosylation (which inactivates telithromycin) and natural mutations in the gene encoding the cellular target (DNA gyrase in the case of ciprofloxacin). As this study is limited to *Streptomyces* and used high concentrations of antibiotics, the full spectrum of antibiotic resistance that may reside in other soil bacteria remains unknown. Another crucial question is whether this reservoir of antibiotic resistance is a source of the genetic elements that confer resistance to pathogens.

V.M. D'Costa et al. (2006). Science 311(5759):374–7.

Antibiotic Resistance: Bridging the Class Divide

Fluoroquinolones are widely used broad-spectrum antibiotics. Bacterial resistance to these drugs is mediated through general mechanisms, such as the upregulation of protein efflux pumps, or specific mutations in the genes encoding the drug targets. Robicsek et al. now demonstrate that bacteria develop resistance to fluoroquinolones by producing an enzyme that inactivates these antibiotics. This is a curious result because fluoroquinolones are completely synthetic antibiotics, not derived from natural compounds made by microbes in the environment. Even more intriguing is the fact that the enzyme that modifies the fluoroquinolone ciprofloxacin—aminoglycoside acetyltransferase—also modifies a completely different class of antibiotics called the aminoglycosides. The investigators found that the *aac(6′)-Ib*-cr gene variant encodes the aminoglycoside acetyltransferase, which inactivates ciprofloxacin. This gene variant, located on a plasmid, renders bacteria resistant to low concentrations of ciprofloxacin. When this gene is combined with another plasmid-encoded gene, *qnrA*, the bacteria become resistant to clinically relevant concentrations of ciprofloxacin. Importantly, plasmids containing these two genes are found in clinical isolates of pathogenic *Escherichia coli*. Two specific mutations in *aac(6′)-Ib*-cr are responsible for bacterial resistance to ciprofloxacin; these mutations do not interfere with the acetylation of aminoglycoside antibiotics. Acetylation of ciprofloxacin, which inactivates this antibiotic, occurs on a specific nitrogen atom that is not present in all fluoroquinolones. Thus, bacteria harboring plasmids containing *aac(6′)-Ib*-cr are not resistant to all fluoroquinolones. However, bacterial resistance to multiple classes of antibiotics provides a strong positive selection pressure and is becoming more common. The next step is to determine the prevalence of the *aac(6′)-Ib*-cr and *qnrA* genes in different bacterial populations and to discern whether there are other examples of inactivation strategies that span different classes of antibiotics.

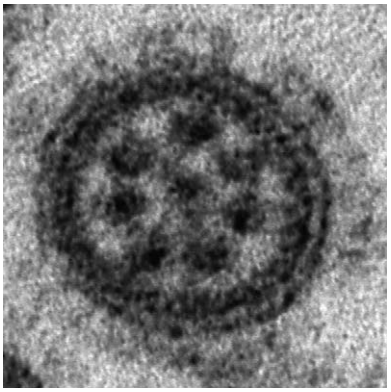
A. Robicsek et al. (2006). Nature Medicine, 12(1):83–8. Published online 20 December 2005.10.1038/nm1347.

Influenza A Virus Genomes in the Spotlight

Obenauer et al. have at their disposal the St. Jude Influenza Repository from which they sequenced 336 strains of avian influenza virus (AIV) from many different bird species and from different regions of the world. Included in this number are examples from all known hemagglutinin (HA) and neuraminidase (NA) serotypes. These two viral surface

glycoproteins are important targets of the host immune system and are the principal components that undergo reassortment between different influenza viruses to create new viral strains. In this study, Obenauer et al. report the analysis of 2196 new AIV gene sequences and 169 complete AIV genomes. This information allowed the authors to further classify the different influenza virus strains. The majority of avian virus genomes exhibit variability in the genes encoding HA, NA, and the nonstructural proteins NS1 and NS2. As HA and NA are targeted by the host immune system, it is not surprising that these components exhibit pronounced variability. The nonstructural proteins encoded by the NS gene are synthesized in AIV-infected cells but are not packaged into virions. The NS1 protein inhibits expression of host antiviral genes that encode interferons, the transcription factor NF-κB, and the protein kinase PKR. By analyzing the NS gene in the sequenced AIV genomes, the authors discovered a previously unrecognized PDZ domain binding motif (PL) in the carboxyl terminus of all avian NS1 proteins. PDZ domains provide a platform for the assembly of signaling molecules. The authors discovered subtle differences in the PL domains of NS1 from avian and human influenza viruses. Synthetic peptides and full-length avian and human NS1 proteins were tested for binding to PDZ domains. Importantly, PL motifs from avian but not human strains of influenza virus bind to the PDZ domains of a variety of human proteins. This may contribute to AIV pathogenicity in humans as the avian NS1 protein may block different signaling pathways in infected human cells. This study provides a wealth of information that can be tapped by researchers analyzing avian influenza viruses that may have pandemic potential.
J.C. Obenauer et al. (2006). Science. Published online 26 January 2006, 10.1126/science.1121586.

Getting to the Heart of the Virion



Electron micrograph showing the interior of an influenza A virus particle. The viral RNA-protein complexes have a distinct organization in each virus particle with a central complex surrounded by seven others. Image courtesy of Yoshihiro Kawaoka.

Understanding the basic organization of the influenza A virus may provide clues to the best strategies to combat this pathogen. In their new study, Noda et al. longitudinally and transversely sectioned influenza A virions budding from MDCK-cultured cells 10 hr postinfection and analyzed these sections by electron microscopy. The authors show that Influenza A virions appear to include a set of eight rod-like structures (see figure). This organization was observed in all tested strains: both spherical and filamentous (as seen in newly isolated strains). Using immunoelectron microscopy, the investigators showed that the particles correspond to ribonucleoprotein complexes (RNPs). These results address a controversy in the field of influenza research, namely whether the RNPs are packaged randomly or in an ordered fashion. Combined with previous studies indicating that the RNPs have segment-specific packaging signals, an organized packaging model seems likely. These signals must be fairly well-conserved, and perhaps molecules that interfere with the packaging of RNPs may represent a new therapeutic opportunity.
T. Noda et al. (2006). Nature, 439(7075):490–2.

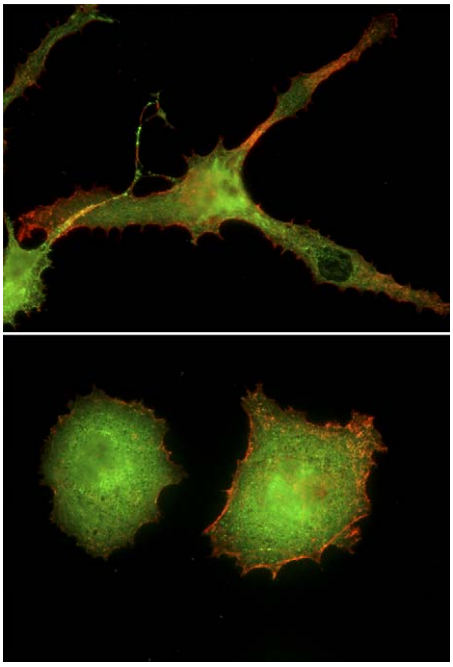
siRNAs: A Promising Alternative to Vaccination?

Palliser et al. use siRNAs to prevent transmission of lethal herpes simplex virus 2 (HSV-2) in mice. First, they established that siRNAs were taken up by the vaginal epithelial cells of mice when they administered fluorescently labeled siRNAs mixed with a transfection reagent into the mouse vagina. These siRNAs silenced gene expression for over a week throughout the genital tissue. Next, they showed that siRNAs designed to target the degradation of three essential HSV-2 mRNAs reduced viral replication and virion production in fibroblasts cultured in vitro. One particular siRNA called U29.2—which is complementary to the sequence of a viral DNA binding protein—was the most effective at suppressing viral replication in cultured cells. The authors then tested whether these siRNAs could protect mice from HSV-2 infection. Seventy-five percent of animals pretreated with UL29.2 and then infected with HSV-2 survived, compared to only 25% of control animals. When pretreated with another siRNA, UL27.2 (which targets degradation of the HSV-2 viral envelope glycoprotein), 60% of mice infected with HSV-2 survived. Shedding of HSV-2 from the mouse vaginal epithelium was measured 6 days after infection. All infected control mice shed virus on day 6, but the virus could not be detected in 70% of UL29.2 siRNA-treated mice or in 50% of UL27.2 siRNA-treated animals. The cervico-vaginal mucosa, which is damaged during HSV-2 infection, appeared healthy in the siRNA-treated mice. When animals were treated with siRNAs 3 or 6 hr after infection with HSV-2, only a combination of UL29.2 and UL27.2 succeeded in protecting them (5 of 6 mice survived). Palliser and colleagues provide a compelling study that demonstrates the potential use of siRNAs to treat or prevent sexually transmitted viral infections. Importantly, treatment of mice with siRNAs after infection with HSV-2 also was effective. Although many

aspects of using siRNAs to treat viral infections—such as how long protection lasts, methods for easy administration of siRNAs and cost—need to be worked out, this study is an important step forward in our strategies to combat viral pathogens.

D. Palliser et al. (2006). Nature. 439(7072):89–94. Published online 23 November 2005, 10.1038/nature04263.

Molecular Mimicry Strikes Again



Infection of cultured cells with the wild-type WR strain of vaccinia virus (top) but not the highly attenuated MVA strain (bottom) causes the cells to migrate and extend projections. Images courtesy of Michael Way.

Valderrama et al. compared the wild-type vaccinia virus (Western Reserve; WR) to the attenuated modified vaccinia Ankara (MVA) strain to understand why the WR strain causes infected cells to migrate and to extend projections, whereas the MVA strain does not (see figure). The authors were able to attribute this difference to the F11L gene as expression of this gene in the MVA virus induced migration and morphological changes in infected cells. Expression of this gene in uninfected cells caused a decrease in actin stress fibers. This phenotype is similar to that observed after expression of a dominant-negative version of the small GTPase RhoA. Interestingly, F11L blocked stress fiber formation induced by a constitutively active version of RhoA, indicating that F11L might inhibit signal transduction pathways mediated by RhoA. RhoA and F11L interacted both in vitro and in infected cultured cells, and the interaction is mediated by the C terminus of F11L. On closer inspection, the authors noticed some homology between amino acid residues in this C-terminal region of F11L with a domain in the Rho-associated kinase ROCK. Furthermore, these residues in ROCK mediate the interaction with RhoA. Mutations in this region of F11L affected the interaction with RhoA and did not stimulate the production of cell projections when expressed in MVA-infected cells. The authors go on to show that F11L interfered with the ability of RhoA to interact with ROCK as well as with mDia, another regulator of actin organization. F11L is highly conserved among the orthopoxviruses and is essential for the production of virus particles. This key protein in important for viral assembly as well as cell motility and may be a good target for developing drugs to limit the spread of orthopoxviruses.

F. Valderrama et al. (2006). Science 311(5759):377–81.

Priya Prakash Budde