structural proteins involved in early phases of the replication cycle; and (ii) the unique mechanism of action of these compounds is complementary to that of other anti-HCMV agents that do not target DNA replication, such as the indolizine-1-carboxamide MCV423, which acts at a stage of the replication cycle that precedes the DNA polymerase step, and the L-ribose benzimidazole derivative 1263W94 (marivavir), which appears to be targeted to the UL97 protein kinase (currently undergoing clinical trials) [4].

1 Bloom, J.D . *et al.* (2003) Thiourea inhibitors of herpes viruses. Part 1: bis-(aryl)thiourea inhibitors of CMV. *Bioorg. Med. Chem. Lett.* 13, 2929–2932

2 Jones, T.R. *et al.* (2004) Specific inhibition of human cytomegalovirus glycoprotein B-mediated fusion by a novel thiourea small molecule. *J. Virol.* 78, 1289–1300

3 Bloom, J.D. *et al.* (2004) Thiourea inhibitors of herpes viruses. Part 2: *N*-Benzyl-*N*′-arylthiourea inhibitors of CMV. *Bioorg. Med. Chem. Lett.* 14, 3401–3406

4 De Clercq, E. (2003) New inhibitors of human cytomegalovirus (HCMV) on the horizon. *J. Antimicrob. Chemother.* 51, 1079–1083

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Biology

Microbiology

Caged and GRABbed: *Streptococcus pyogenes* protease degrades antimicrobial peptide

Regulation of proteolysis is crucial in maintaining balance in the human body and is accomplished by a complex array of proteases and protease inhibitors. Many infections are characterized by uncontrolled proteolysis leading to tissue damage and activation or inhibition of proteins involved in the immune defense and coagulation. The human pathogen *Streptococcus pyogenes* secretes several proteases that degrade antibodies, complement and coagulation factors. The streptococcal cysteine proteinase, SpeB, degrades several such molecules including the antimicrobial peptide LL-37, a component of the innate immune defense. In addition to proteases, *S. pyogenes* expresses a surface protein, GRAB, that binds the human proteinase inhibitor α2-macroglobulin (α2M) and thereby controls proteolysis at the bacterial surface.

Nyberg *et al.* [1] have now shown that SpeB is trapped in the α2M cage forming a SpeB-α2M complex with no activity on larger proteins. On the contrary, SpeB-α2M allows the small peptide LL-37 to enter the cage and even more efficiently inactivates LL-37 than SpeB alone. By comparing wild type bacteria and a mutant lacking GRAB expression, it was shown that SpeB-α2M complexes bound to bacteria protects them from killing by LL-37. This protection is dependent on all three active components, SpeB, α2M, and GRAB.

This study elegantly demonstrates an intricate process where S. pyogenes through the surface protein GRAB acquires the proteinase inhibitor α2M to trap its own proteinase SpeB at the bacterial surface. This allows the bacteria to direct the proteolysis towards small molecules, such as antimicrobial peptides, and concentrates the activity to the bacterial surface where it is needed the most.

1 Nyberg, P. *et al.* (2004) α2-macroglobulin-proteinase complexes protect *Streptococcus pyogenes* from killing by the antimicrobial peptide LL-37. *J. Biol. Chem.* doi:10.1074/jbc.C400485200 (EPub. ahead of print; http://www.jbc.org)

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Pseudomonas affects *Candida* differentiation

The ability of *Candida albicans* to reversibly switch between yeast and hyphal morphologies plays an essential role in the pathogenesis of infections caused by this opportunistic pathogen. Earlier work has shown that co-cultivation of *C. albicans* with the bacterial pathogen *Pseudomonas aeruginosa* results in killing of hyphae, whereas yeast are spared. A current study by the same group now demonstrates that one of the main quorum-sensing molecules secreted by *P. aeruginosa* can inhibit the yeast-to-hypha transition in *C. albicans*. [2].

To screen for interactions between these two organisms, Hogan *et al.* developed a plate assay utilizing a *HWP1-lacZ* derivative of *C. albicans*, hyphae-inducing agar, and the chromogenic substrate X-Gal. As yeast convert to hyphae they express *lacZ* (*HWP1* is only expressed in hyphae) and turn the agar blue. When wild-type *P. aeruginosa* was grown on these plates, the bacterial colonies were surrounded by white halos that contained only yeast forms. Further studies with bacterial mutants and purified compounds revealed that this effect was due to secretion of the quorum-sensing molecule, 3-oxo-dodecanoyl-homoserine lactone (3OC12HSL).

RT-PCR analysis confirmed that this compound was capable of suppressing hypha-specific genes and inducing yeast-specific genes. Other C12 compounds, such as dodecanoyl-HSL and dodecanol, also inhibited filamentation whereas the corresponding C10 and C14 derivatives had no effect. Farnesol, which also has a 12 carbon backbone, has been previously shown to be a quorum sensing molecule secreted by *C. albicans* that suppresses the yeast to hyphae transition. It is not known how farnesol and 3OC12HSL inhibit filamentation or whether they act at the same site.

The ability of *P. aeruginosa* to affect *C. albicans* differentiation *in vitro* could provide insight into the growth and survival of these organisms within polymicrobial communities in colonized patients.

PII: S1359-6446(04)03193-9

2 Hogan, D.A. et al. (2004) A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. Mol. Microbiol. doi:10.1111/j.1365-2958.2004.04349.x (E-pub. ahead of print; http://www.blackwell-synergy.com)

Targets

Drug screening of membrane proteins by mass spectrometry

Mass spectrometry has rapidly gained acceptance in the screening of combinatorial and small molecule libraries using limiting amounts of protein targets. However, the application of these methods has been limited to the use of soluble protein targets. However, the majority of validated drug targets are membrane proteins, which are not readily soluble and require tedious preparation, making them not amenable for HTS.

Maintenance of the native state of membrane proteins requires its incorporation in the appropriate lipid environment. Mass spectrometry has been used to study lipid–protein interactions but never in a functional state. Ilag *et al.* now demonstrate for the first time the observation of the functional interaction of a protein micelle complex of EmrE from *Escherichia coli* solubilized with the detergent dodecylmaltoside (DDM) while maintaining drug binding within the complex in the gas phase [3]. This was made possible through the use of a modified mass spectrometer that can measure high mass complexes.

EmrE is a 110-amino acid transmembrane protein involved in multidrug transport and its recent quaternary structure determined by cryoelectron microscopy reveals an asymmetric dimer. High-resolution structures of the subunits are available and one substrate molecule, tetraphenyl phosphonium (TPP+) binds simultaneously to each subunit of the EmrE monomer. Electrospray mass spectrometric analysis of the complexes revealed a broad peak that contains protein, lipid, detergent and substrate, that can be dissociated upon increase of the collision voltage with the corresponding interactions directly correlated to the level of voltage required for dissociation. Interactions between protein and drug required higher voltage, whereas dissociation of lipid molecules required lower collision cell voltage. Mass spectrometric measurements of complexes in the gas phase were consistent with interactions observed in solution such as the binding conditions for TPP+ to EMrE. This study highlights the potential of using mass spectrometry for screening ligands (small molecules and large molecule (protein) ligands) against membrane proteins.

3 Ilag, L.L. *et al.* (2004) Drug binding revealed by tandem mass spectrometry of a protein-micelle complex. *J. Am. Chem. Soc.* 126, 14362–14363

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Cancer biology

'Magic bullets' in the war on cancer go live

In warfare, recognizing your adversary's weak spots is key to success. Judah Folkman is credited for being the first to realize that in cancer, metastases must recruit blood vessels. Without proper vascularization, tumors cannot grow beyond just a few millimeters in size. This concept is now the basis of therapeutic strategies that try to block vascular endothelial growth factor (VEGF) with decoy receptors, or that use drugs to inhibit the VEGF receptor kinase. In parallel, the process by which cells protect themselves from poor oxygenation, as orchestrated by the hypoxia-inducible factor HIF-1, is being investigated for antitumor targets.

In yet another variation on this theme, the lab of Bert Vogelstein has been exploiting obligate anaerobic bacteria that will only grow in poorly oxygenated tumors. *Clostridium novyi* spores from which the α-toxin gene had been deleted were injected into tumor-bearing test animals, and this treatment showed efficacy when combined with chemotherapy and irradiation. Such experiments are usually performed in immune-suppressed ('nude') mice, which support the proliferation and testing of a variety of mouse and human tumors. But new work [5] shows that injection with engineered Clostridium in normal, immunocompetent animals is dramatically more effective: even in absence of chemotherapy or irradiation, remissions of up to 30% were observed in mice, rats and rabbits, even where large tumors were involved. Apparently the presence of *Clostridium* 'danger signals' evokes a very potent immune response from which the tumor cannot escape.

Although it remains to be seen if such effective responses can be reproduced in human patients, the authors cite anecdotic evidence, over hundred years old, of cancer patients that went into remission after they had suffered (but survived) postoperative infections. This elegant *Clostridium*-based procedure to recruit the immune system for tumor rejection illustrates another important war lesson: don't engage without the full support of your allies.

5 Agrawal, N. *et al.* (2004) Bacteriolytic therapy can generate a potent immune response against experimental tumors.' (2004) *Proc. Natl. Acad. Sci. U. S. A.* 101, 15172–15177

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HIV-1 CXCR4 coreceptor reduced to silence

HIV infects CD4+ target cells via a specific receptor-mediated fusion event between the retroviral envelope glycoproteins and the CD4 receptor. Chemokine receptors CXCR4 and CCR5 also participate closely in viral fusion and entry into cells. Current models suggest that the initial interaction between the viral envelope protein gp120 and CD4 on target cells results in a conformational change in gp120 that exposes the coreceptor binding site. After binding to the coreceptor molecule, additional conformational changes allow the viral gp41 protein to initiate fusion.

Zhou N. *et al.* have now investigated the potential use of small interfering RNAs (siRNAs) targeted to CXCR4 to inhibit HIV-1 fusion [4]. SiRNAs are double-stranded RNAs of 21–25 nucleotides that can silence genes in a sequence-specific manner and at a posttranscriptional level. The authors use siRNAs with homology to a motif in the mRNA encoding for CXCR4 chemokine receptor. They first show by fluorescent microscopy and fluorescence-activated cell sorting (FACS) that the expression of CXCR4 is downregulated at the surface of cells transfected with the specific siRNAs. This inhibition is specific, as siRNAs targeting CXCR4 have no effect on the expression level of CCR5 and CD4, and because the use of siRNAs targeted to other chemokine-receptor sequences has no effect on CXCR4 expression level.

Next, the authors evaluated the ability of CXCR4 siRNAs in blocking CXCR4- and HIV-1 gp120-mediated cell membrane fusion, using a luciferase-based cell–cell fusion assay. Transfection of CXCR4 siRNAs resulted in the specific inhibition of CXCR4 coreceptor activity, both for CXCR4-tropic and dual-tropic (CXCR4 and CCR5) HIV-1 isolates. SiRNAs targeting CXCR4 mRNA had no effect on CCR5 coreceptor activity. CXCR4 siRNAs can inhibit cell-free virus infection but less effectively than cell–cell fusion.

This study shows the potential use of siRNAs as a therapeutic approach to alter the infection process of human cells by some HIV-1 strains. Whether this approach will be efficient in inhibiting HIV-1 infection in peripheral blood, lymphoid tissue and other sites like the central nervous system remains unknown. Finding a suitable and efficient *in vivo* siRNAs delivery system to treat HIV-1 infected patient will also be required.

4 Zhou, N. *et al.* (2004) Inhibition of HIV-1 fusion with small interfering RNAs targeting the chemokine coreceptor CXCR4. *Gene Ther.* 11, 1703–1712

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Business

Collaborations

Agilent Technologies and ExonHit Therapeutics to collaborate

Agilent Technologies (http://www.agilent.com) and ExonHit Therapeutics (http://www.exonhit.com), a private drug discovery company, have announced a research collaboration to combine Agilent's microarray platform and ExonHit's alternative RNA-splicing technologies and expertise. This collaboration explores the development of a microarray-based solution that will enable scientists to properly monitor the expression of splice variants.

Splice variants are variable sequences of RNA produced from the same gene in DNA, resulting in the creation of different proteins potentially affecting cellular regulation. Scientists developing therapeutics are increasingly interested in this emerging field as the expression of splice variants can provide novel targets, could indicate disease states, and can be altered by exposure to drugs and toxins.

Agilent and ExonHit are working together to optimize microarray design, reagent protocols and data analysis methods for splice variant studies. As a pioneer in alternative RNA splicing, ExonHit realized that the proper characterization of splice variant expression required dedicated profiling platforms. The company has received notice of the allowance of its patent, which broadly claims nucleic acid arrays that enable the detection of alternative RNA splicing events via either intron or exon and splice junction-specific probes.

Initial results from an experimental splicing array of G-protein coupled receptors, designed by ExonHit and produced by Agilent pursuant to the collaboration, were presented at Splicing 2004, an annual symposium on alternative RNA splicing, by Richard Einstein, Vice President of R&D North America at ExonHit. The array detected multiple isoforms of several genes, and showed good reproducibility and specificity. The companies are expected to work with early test sites to generate additional experimental results.

Sigma-Aldrich and Procognia commercialize functional human protein arrays

Sigma-Aldrich Corporation (http://www.sigmaaldrich.com) and Procognia (http://www.procognia.com) have an exclusive agreement that calls for Sigma-Aldrich to market novel arrays of functional human proteins developed by Procognia. This agreement will significantly expand Sigma-Aldrich's pipeline of products for the advancement of proteomic research.

Procognia has developed a proprietary tag technology to create the first arrays of biologically-related human proteins that retain their native functions in the array format. The first protein array in the pipeline of products will contain wild-type human p53 and its important germline SNP variants. Procognia's functionally arrayed SNP variant proteins will be the first tool that enables researchers to investigate the mechanism of cancer progression on many proteins in parallel.

David Harvey, Chairman and CEO of Sigma-Aldrich, remarked, 'I am pleased that Sigma-Aldrich will have the opportunity to exclusively market a product with the power to finally deliver functional arrays of human proteins. This proprietary technology represents a significant advancement over existing protein array technologies, which do not orient nor confer functionality of proteins on the array.'

Ron Long, Procognia's CEO and former CEO of Amersham Pharmacia Biotech, said, 'Sigma-Aldrich is emerging as the strongest force in leading edge proteomic tools for the life sciences market today, and I feel that they are the best partner for Procognia's protein function arrays. With the human p53 SNP arrays, we will mark a first in functional protein array development and open the door for rapid uptake of the future protein array products that will follow.'

IMS announces agreement with European Generic Medicines Association

IMS Health (http://www.imshealth.com) today announced an exclusive data and knowledge sharing agreement with the European Generic medicines Association (EGA; http://www.egagenerics.com) to advance the quality of healthcare decision-making by government and industry

1359-6446/04/$ – see front matter PII: S1359-6446(04)03178-2