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The study of antiviral drug resistance is an expanding universe. What was once a small community of biochemical virologists now includes biophysicists, molecular and cellular biologists, mathematical modellers, pharmacologists and clinicians. One inevitable outcome of this explosion is that critical studies in drug resistance are often presented at large impersonal conferences or at highly specialized meetings. Opportunities for principal investigators, fellows and students to present new findings and debate established paradigms in a forum with broad perspective and intimate format are limited. Such an opportunity, the First HIV DRP Symposium ‘Understanding Antiviral Drug Resistance’ (Chantilly, Virginia, USA, December 3–6, 2000), was organized by John Coffin of the National Cancer Institute’s HIV Drug Resistance Program, and John Mellors of the University of Pittsburgh. The Symposium programme and links to abstracts can be viewed at http://www.ncifcrf.gov/hivdrp/Symp2000_program.html

The broad scope of the meeting was set by an opening session in which the three great drug-resistance problems of clinical significance were reviewed: antibiotic resistance in bacteria (Julian Davies); drug resistance in viruses (John Mellors); and chemotherapy resistance in cancer cells (Michael Gottesman). These sobering reminders of the limitations and multiple mechanisms of drug therapy failure were tempered by the increasing number of new targets for viable treatment strategies. In addition, better understanding of the mechanisms of drug failure will help to design effective and potent strategies.

New findings were discussed in poster and platform presentations from a host of international academic, government and pharmaceutical laboratories.

New and emerging targets

New inhibitors of HIV entry that block HIV co-receptor binding and fusion are currently in clinical trials, but the development of resistance to these entry inhibitors is poorly understood. S Kuhmann described escape mutants of HIV in peripheral blood mononuclear cell cultures after prolonged exposure to the CCR5 binding inhibitor AMD101. Surprisingly, these mutants continued to use CCR5 as a co-receptor even though CXCR4 was available for binding; mutants were cross-resistant to RANTES and to TAK779, and exhibited more efficient binding to CCR5 in the presence of AMD101. Numerous mutations were identified in the resistance-conferring env gene, but the precise residues responsible for resistance remain uncertain. J Kappes described mutants with high-level resistance to the fusion inhibitor T-20, which were selected in vivo during early monotherapy trials with relatively low doses of the drug. Several single and double mutations in the gp41 GIV motif were essential for fusion activity; single and double mutations in GIV were capable of conferring ≥ 8-fold increase in IC₅₀. The role of additional mutations that were identified outside this domain in the development of resistance remains uncertain.

Reports on new drug development included a presentation by A Patick, who described initial data on the inhibition of picornavirus (human rhinovirus, HRV) replication using the protease inhibitor AG7088; the picornavirus protease is unrelated to other known human proteases, suggesting that inhibition of the HRV enzyme may be relatively selective. Strict conservation of amino acid residues in HRV protease involved in AG7088 inhibition suggests the drug may have activity in all serotypes. AG7088-induced suppression was present in lab strains or clinical isolates with clinically achievable EC₅₀. Resistant mutants have not yet been identified. The possibility that there may soon be two drugs (AG7088 and pleconaril) with activities against entry and maturation steps in HRV replication is likely to improve our understanding of these viruses and permit new therapeutic strategies.

Several findings from basic research laboratories suggested new targets for therapy. S Campbell described the intriguing finding that inositol phosphates (IPs) are active as co-factors in promoting full-sized HIV capsid assembly in vitro. It is uncertain which IPs promote capsid formation in cells, but given the central role of IPs in signal transduction pathways, their results may broaden understanding of the regulation of late steps in HIV replication.

R Krug presented data targeting the cap-snatching activity of influenza A and, in particular, addressed the novel therapeutic potential of targeting interactions between influenza
virus protein NS1, cellular RNA-binding proteins PAB II and CPSF, and virus mRNA.

A Bashirova described the discovery of a new lectin, L-SIGN, which is related to DC-SIGN and is capable of binding HIV and enhancing infection. L-SIGN is expressed in liver but is absent from dendritic cells, and the authors speculated that L-SIGN may play an essential role in immune cell trafficking through the liver, and perhaps in presenting HIV to migrating cells.

Information about HIV gene products may provide new targets for antiviral therapy. EO Freed presented new data indicating that cholesterol-rich membrane rafts are involved in intracellular transport of HIV Gag proteins to cell surfaces; inhibition of cholesterol biosynthesis by 3-hydroxy-3-methylglutaryl co-enzyne A reductases using FDA-approved statins reduced HIV replication in cell culture. A Rice extended his work on the cellular factor cyclin T1, which is necessary for Tat transactivation of HIV transcription, by describing the function of cyclin T1 in primary monocytes and macrophages. RJ Gorelick presented ongoing studies characterizing the role of nucleocapsid in HIV replication. U Schubert discussed the role of ubiquitination in HIV-1 Gag processing and described reductions in virus release by proteasome inhibitors.

**New approaches to old targets**

Several discussions expanded on the understanding of established agents. Detailed understanding of resistance to zidovudine and stavudine has remained a critical unresolved problem for investigators studying HIV. Recent studies demonstrating the presence of a pyrophosphorolysis-mediated excision activity enabling reverse transcriptase (RT) to remove terminal-incorporated residues (including zidovudine) have not provided a detailed molecular mechanism to explain the suite of mutations conferring high-level zidovudine resistance and increased excision activity. SH Hughes presented data from X-ray crystallographic, enzymatic, and mutational studies to derive a molecular model explaining zidovudine resistance-conferring mutations as a result of their involvement in binding an ATP molecule required as the pyrophosphate donor for excision. The model suggests that the azido moiety of zidovudine causes strands to stall after zidovudine incorporation, increasing the time spent in the active site and thus the probability of excision. E Matsuura and co-workers from the Scott laboratory suggested that the excision mechanism is inhibited by the presence of high concentrations of dNTP corresponding to the next nucleotide to be incorporated. Inhibition of excision was more pronounced for stavudine and minimal for zidovudine. These authors speculated that the difference may explain, in part, the discrepancy observed between the degree of resistance to zidovudine and stavudine measured in phenotyping assays, which are performed in tissue culture cells that have relatively high intracellular dNTP concentrations. A Mas described recent findings that a phosphorolytic excision mechanism contributes to resistance in the nucleoside RT inhibitor multidrug-resistant 69 (S–S) insertion mutant of HIV.

Other RT studies included presentations by Hu and colleagues characterizing sequence size and homology requirements involved in RT-mediated strand transfer. These studies are critical to understanding the molecular events of reverse transcription, but also relate directly to understanding retroviral recombination, a process that is likely to figure prominently in the acquisition of new drug-resistant mutations.

Characterizing protein–protein interactions may yield effective new inhibitors to established targets. DA Davis presented developments in identifying peptides capable of destabilizing HIV protease heterodimer formation; such research may yield useful new agents that avoid the problem of protease inhibitor cross-resistance. G Tachedjian and Goff presented data suggesting that non-nucleoside RT inhibitors (NNRTIs), including efavirenz and nevirapine, enhance RT heterodimer formation in yeast two-hybrid and GST fusion assays. Dimerization-defective mutants L234A and W401A, which by themselves were non-functional in the two-hybrid system, were rescued in the presence of efavirenz. The mechanism for this activity, which was not shared by the less potent NNRTI delavirdine, remains uncertain. Interestingly, the non-nucleoside TSAO group of RT inhibitors, which are believed to have a binding site that overlaps the site for nevirapine and efavirenz, have been recently shown by M Parniak and co-workers to destabilize RT heterodimer formation. It seems likely that clarification of how these agents affect heterodimer formation may lead to novel strategies to inhibit RT function.

Ribavirin has over 20 years of clinical experience with an expanding range of susceptible viruses, with the recent addition of the flaviviruses hepatitis C and West Nile virus. Ribavirin has been reported to potentially affect numerous steps in replication, from nucleotide metabolism to transcription and capping, but the precise mechanism of ribavirin activity in some virus systems remains uncertain. In inhibiting poliovirus replication, CE Cameron demonstrated that ribavirin was an effective mutagen, with 10-fold increases in G–A and C–U transversion rates, and suggested that it drives poliovirus to error catastrophe. Although the concentrations of ribavirin used to obtain this result have not been achieved clinically, the possibility of manipulating replication fidelity to perform lethal mutagenesis, as suggested for HIV by Loeb and Mullins, is intriguing.

Discussions of retroviral RT RNase H inhibitors highlighted key structural determinants, including divalent cation chelation by hydrazones (J Peliska) and resonance-stabilized planar configuration by Fe chelators for selective binding to RNase H (M Parniak). SFJ Le Grice described application of photoactive coumarin derivatives to identify sites of drug contact; such reagents will be especially useful in understanding the mechanism of catalytic activity and of inhibition by the coumarin class of agents. A Campbell broadened understanding of the versatility of viral and...
bacterial RNase H by demonstrating that murine leukaemia virus (MuLV) RNase H could rescue growth of Escherichia coli defective in RNase H. Application of novel high-throughput screening techniques by the SFJ Le Grice group may rapidly identify new RNase H inhibitors for additional study.

**Virus evolution**

A session on evolution permitted one of the conference organizers, John Coffin, to present a primer on the principles of population genetics as applied to virus replication. JM Coffin stressed that measuring certain parameters, especially the proportion of the total virus population participating in replication (effective population size, Ne), was essential in determining how new mutations will be fixed in the population. Large effective populations indicate that mutants will be fixed in a predictable, deterministic fashion, while mutations in small Ne populations will become fixed in a stochastic manner. I Rouzine and JM Coffin described the theoretical behavior of populations with intermediate Ne (selection-drift) in detail. R Ribeiro followed with a comparison of theoretical stochastic and deterministic models of HIV replication; both simulations suggest that resistance mutations are more likely to be present prior to therapy than to be generated after therapy is introduced.

Attempts to translate these models to HIV replication in human populations remain preliminary; linkage disequilibrium analyses and other methods have been utilized to estimate Ne, although the appropriate in vivo data sets to address this issue remain limited. Data sets to measure the selective advantage of mutant viruses remain equally challenging. R Swanstrom, however, was able to detail the step-wise increase in relative replicative capacity during development of resistance to ritonavir by utilizing a sensitive heteroduplex-tracking assay. These findings and the number of preliminary studies of relative replication capacity (reported by J Balzarini, G Garcia-Lerma, TL Loftus and S Palmer) illustrate that the study of selection per se will continue to be a growth industry.

The lack of information regarding HIV evolution in vivo is especially unfortunate for paediatric HIV populations. Relatively high viral loads and the presence of an immature immune system and highly active thymus are characteristics that are likely to have substantial implications for HIV replication in children that are not readily modelled from studies in adults. In an attempt to address this void, Persaud studied a series of paediatric patients undergoing drug therapy and found that the small amount of HIV expressed in highly suppressed patients often consisted of genotypically wild-type, not drug-resistant, HIV. Ongoing studies to determine whether these wild-type HIV species are evolving during suppression are critical to understanding the nature of HIV reservoirs in this patient population.

**Assay development**

New studies in assay development included further advancements in yeast-based NNRTI phenotyping (DV Nissley), and initial studies of HIV protease phenotyping using in vitro protease assays employing virus-like particles (M Iga) or coupled transcription–translation systems (Y Yokomaku). HIV genotyping via microarray was reported by Roche / Affimetrix scientists, and J Kappes described cell lines capable of phenotyping primary HIV isolates; further experience to compare sensitivity, specificity and throughput of these assays will be welcome. H Isom described advancements in addressing the frustrating limitations in cultivating hepatitis B virus (HBV) in vitro: applying baculovirus technology to permit long-term expression of HBV and sensitive assays to detect HBV virion and replicating DNA, this system is suitable for detailed studies of drug action and for clinical application for HBV drug-resistance phenotyping.

**Review sessions**

The working sessions were punctuated with state-of-the-art talks reviewing the development of resistance in herpesviruses (K Biron, PG Spear), orthomyxoviruses (GM Air), paramyxoviruses (RA Lamb), hepadnaviruses (WS Mason, S Locarnini), picornaviruses (MG Rossmann), and HIV (JP Moore, SH Hughes, WI Sundquist, D Kempf, SFJ Le Grice, D Hazuda). The review sessions highlighted the profound impact of modelling studies based on crystallography and NMR structural data in understanding protein function and drug inhibition. The elegant studies Rossmann described in defining the structure of VP1 canyons were essential to understanding the mechanisms of resistance to the binding inhibitor pleconaril. Moore described how identification of the TAK 779 binding site on CCR5 was predicted from modelling studies, but was not evident after an exhaustive alanine-scanning mutation; crystallography played essential roles in development of ABT-378 to inhibit HIV protease (D Kempf) and of AG7088 to inhibit HRV protease (A Patick).

The review sessions permitted direct comparisons of different virus systems. Common threads regarding mechanisms of resistance to therapy were evident, such as mutations that make a drug-binding cavity larger (HRV/pleconaril and HIV/NNRTI). Some similarities might be anticipated, such as the analogous mutations conferring resistance to lamivudine in HBV and HIV (S Locarnini), or the similar mutations identified in HIV and feline leukaemia virus protease after protease inhibitor exposure (Z Beck). More often than not, however, comparisons of virus resistance revealed intriguing discrepancies. For example, MuLV RT is naturally resistant to lamivudine and has the amino acid sequence YVDD at the position corresponding to
YMDD in HIV; this result may be expected, since in HIV, the M–V mutation confers resistance. However, when V Pathak and co-workers reconstructed MuLV RT with the YMDD motif, the resulting virus was still highly resistant to lamivudine, suggesting that regions outside the YMDD motif may contribute to lamivudine resistance. In this regard, S Locarnini identified a new mutation outside the YMDD motif of HBV that improved replication of the lamivudine-resistant virus (containing the YVDD or YIDD motif). Such presentations illustrated the difficulties in attempting to predict resistance profiles, infer the relative importance of individual mutations, or extrapolate between virus systems. With the number of new drugs in the various development pipelines, it is likely that all of the technological tricks presented at this meeting (and more) will be necessary to identify and characterize new escape mutants.

Owing to the broad perspective and the cross-fertilization potential, meetings of this type are very likely to become habit-forming (the next Symposium is already in the planning stage). Such gatherings serve an essential function in preventing the field of drug resistance from expanding into numerous parallel universes.

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