With over 350 million chronic carriers of the hepatitis B virus (HBV) worldwide and the realization that chronic carriage leads to the clinical sequelae of liver cirrhosis and hepatocellular carcinoma, control of hepatitis B has emerged as one of the significant public health challenges of the decade. The implementation of universal infant immunization has already had an effect on the burden of chronic liver disease, but what is to be done with the existing pool of carriers?

In most countries, the only currently licensed treatment is interferon-α but this is effective in less than a third of selected patients. Important factors in the therapy of a chronic infection such as HBV should include an understanding of the natural history of the disease, the appropriate use of laboratory investigations and when to intervene, and appropriate measurement of the response to a therapeutic agent including the effect(s) on both the host and the virus and the cost–benefit to the community.

The majority of the world’s carriers acquired HBV at birth or shortly thereafter. For the next two or three decades, most individuals are then in a ‘tolerant’ phase, with high levels of viral replication and minimal disease activity. Carriers then enter the ‘immuno-elimination’ phase where disease activity increases owing mainly to attempts by the host’s immune system to clear virus-infected hepatocytes from the liver. During this phase, the viral load decreases and there are often hepatic flares before HBV (as measured by viral DNA) is eventually eliminated from the blood compartment. The hepatitis B e antigen (HBeAg) becomes undetectable and HBeAg-specific antibodies (anti-HBe) appear. Interferon-α is only effective when used to treat carriers who are in this immuno-elimination phase but, even in this narrow therapeutic window, HBeAg seroconversion occurs in no more than 30% of cases.

The immune-response-driven clearance of HBV can select for escape mutants of the virus (e.g. the pre-core HBV mutant) which may take over from wild-type HBV. The natural history of anti-HBe-positive chronic hepatitis B has recently been reviewed and therapeutic intervention is clearly indicated. Interferon-α provides some benefit during treatment at this stage of hepatitis B, but over 90% of patients will relapse when this treatment stops.

Renewed interest in the treatment of chronic hepatitis B, especially in individuals who are HBeAg-positive and have high levels of HBV DNA but normal ALT (referred to as wild-type disease in the tolerant phase) and anti-HBe-positive chronic hepatitis B (referred to as pre-core mutant disease), has emerged with the discovery that a number of nucleoside analogues which have anti-herpesvirus (e.g. penciclovir–famciclovir) or anti-HIV (e.g. lamivudine) activity, also possess anti-HBV activity. A number of phase II/III clinical trials are currently in progress with these compounds and preliminary results have been published for both lamivudine and famciclovir. It appears that most patients with chronic hepatitis B who are viraemic, decrease their serum viral DNA levels during therapy and some patients even become HBeAg-negative. Unfortunately, almost all patients relapse as soon as therapy is stopped. The reason for this relapse was recognized more than 10 years ago by Omata and colleagues who demonstrated that the transcriptional template of HBV, the covalently closed circular (CCC) or supercoiled DNA form, is generally not affected by treatment with interferon-α. Similarly, hepadnaviral CCC DNA appears unaffected by nucleoside analogue therapy, despite the fact that all other viral DNA forms in the liver are reduced or eliminated (reviewed elsewhere). A possible reason for this failure is that viral CCC DNA has a very long intra-hepatic half-life and, under normal circumstances, is probably as stable as host-cell DNA. Thus, even long-term antiviral treatments fail to eliminate from infected hepatocytes the viral CCC DNA species which continue to persist and maintain their functionality. Thus, the eradication of chronic HBV infection either requires the permanent inactivation of the viral CCC DNA species or demands the elimination of each infected cell.

The viral dynamics of HBV infection have recently been described in a mathematical model by Nowak et al., who calculated that virus was cleared from the blood with a half-
life of 1 day, whilst the half-life of an infected cell was estimated to be 10–100 days, depending on the inflammatory activity within the liver. Assuming the half-life of infected cells to be 10 days, during the active disease phase, this model led to the prediction that 12 months of lamivudine treatment would reduce the total body viral burden by a factor of about $10^{11}$. The authors claimed that such treatment should be sufficient to eradicate HBV from all infected cells and produce a virological cure. On the other hand, during the inactive disease phase of hepatitis B, when the half-life of an infected cell is 100 days, antiviral treatment would have to be continued for many years in order to achieve a comparable reduction in viral load. These authors concluded that monotherapy alone in the active disease setting could be enough to eradicate HBV from all infected cells, whilst some form of adjunct immunotherapy in combination with treatment with a nucleoside analogue may be needed in order to treat inactive disease adequately. 

The calculations of Nowak and colleagues are based on a half-life for HBV in blood of 1 day, but other investigators have reported half-lives for HBV particles ranging from 2 to 3 days. In the active disease model of Nowak et al., a longer half-life for HBV of 2–3 days would significantly extend the projected duration and cost of monotherapy for patients. The mathematical treatment model of Nowak and colleagues will be useful only if its predictions are in accord with the clinical experience. To date, most long-term monotherapies with nucleoside analogues do not seem to be achieving eradication. Why is this the case? Part of the answer can be deduced from studies in which animal models of HBV infection have been used to study the effects of nucleoside analogues on intra-hepatic as well as extra-hepatic markers of viral replication (reviewed elsewhere). From these and the clinical studies described above, four issues for further drug development have arisen: (i) The role of extra-hepatic (lymphoid) and extra-hepatocyte (bile-duct cells) reservoirs of HBV in response to therapy; (ii) the emergence of strains of HBV which are resistant to famciclovir, lamivudine or both; (iii) the resistance of hepatadnaviral CCC DNA to nucleoside analogue as well as interferon-therapy; (iv) overcoming tolerance in the existing pool of hepatitis B carriers.

HBV replicates in cells other than hepatocytes and this extra-hepatic reservoir of virus may be the source of reactivation/exacerbation of disease following successful eradication of virus from infected hepatocytes by antiviral therapy or natural immune clearance. It has been shown that ganciclovir does not appreciably affect the duck HBV load in extra-hepatic sites such as bile-duct cells and pancreatic islets. Thus, these sites plus the lymphoid compartment represent potential reservoirs for seeding of virus back to hepatocytes even while treatment is continuing. The ability of an antiviral agent to inhibit HBV replication in all cells harbouring virus would, therefore, be an important consideration for the development of an effective therapeutic approach in chronic hepatitis B.

Whilst patients are being managed on long-term antiviral therapy, it is important that no drug-resistant species occur as a consequence of such treatment. Hepatitis B virus DNA contains four open reading frames (ORFs) which overlap with each other. The largest ORF encodes the polymerase protein and it overlaps with the core, envelope, and X proteins, but the catalytic and template-binding domains of the polymerase overlap with the antibody-neutralization domain of HBsAg. This is a potentially important consideration with the use of immune-based therapy such as hepatitis B immunoglobulin or therapeutic vaccines. Two recent reports describe development of resistance of the HBV polymerase protein to antiviral nucleoside analogues in liver transplant patients with recurrent hepatitis B infection who were undergoing therapy with nucleoside analogues. Ling and colleagues, who characterized the changes in the HBV polymerase protein which occurred during lamivudine therapy, found substitutions in domain B (template binding) as well as the domain C (catalytic domain) of the polymerase. A ye and colleagues have found similar changes (but only in domain B of the HBV polymerase) in patients on long-term famciclovir therapy. To date, there have been no reports of mutations in immunocompetent patients on nucleoside analogue therapy, but such pressures will probably result in the selection of resistant isolates in this setting also.

The hepatadnaviral covalently closed circular (CCC) DNA within the nucleus has been shown to be organized as a nucleoprotein complex or viral minichromosome with the viral DNA compacted into nucleosomes. In vivo, the hepatadnaviral minichromosome is actually a population of CCC DNAs that is heterogeneous in both topology and composition. Some molecules are fully compacted into nucleosomes, while other topoisomers contain fewer nucleosomes and represent more open chromatin structures. Probably the fully chromatinized molecules are transcriptionally inert and as long-lived as the cellular chromatin. By contrast, the less chromatinized minichromosomes are likely to be the major templates for viral transcription, and because of their more open chromatin structure may well be more susceptible to degradation and thus less stable molecules in situ. It is noteworthy that one study with duck HBV reported that CCC DNA in explanted hepatocytes displayed a short half-life of only 3–5 days. Thus, future therapeutic approaches will need to address the activity and stability of the hepatadnaviral CCC DNA molecules and find ways either to destabilize and eliminate these molecules for a virological cure or to render them transcriptionally inactive, abating the expression of viral antigens and perhaps achieving a remission of disease.

The pathogenesis of liver injury in chronic hepatitis B, although closely related to ongoing active viral replication, is determined primarily by the host's immune response.
responses. The major cytotoxic T-cell response to HBV appears to be directed to key epitopes of the viral core and HBcAg. This activity has been identified as an important determinant of viral clearance and cell damage. The HBcAg is a toleragen and the aim of removing the tolerogenic effect of circulating serum HBcAg by chemosuppression to induce endogenous immuno-elimination may prove a useful strategy for future management of chronic hepatitis B. Nowak et al. highlighted the need for an immune modulator plus an antiviral agent in combination in order to shorten the treatment of the inactive phase of hepatitis B. The effects of cytokines such as interleukin 2, both on the viral life-cycle as well as on the host's immune response, clearly require further exploration. 

From the preceding discussion, it is clear that no single drug possesses all the properties required of an ideal antihepadnaviral agent and a strong case can be made for combination chemotherapy. For example, there is no existing drug capable of effectively blocking viral replication in all infected cell-types as well as inhibiting viral CCC DNA generation and processing. Furthermore, the therapeutic protocol should also include agents which can modify the ineffective immune response to HBV-infected hepatocytes. Finally, the combination regimen should substantially reduce the risk of virus developing resistance. In this way, we should be in a position to develop a therapeuetic protocol that can successfully control HBV and reduce the global viral burden of this important pathogen with its associated morbidity and mortality.

References

The role of macrolides in *Streptococcus pyogenes pharyngitis*

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The particular pattern of antibiotic prescribing adopted by Italian physicians in the therapy of community-acquired respiratory infections,\(^1\) and the ensuing selective pressure, have been held responsible for the lower incidence of resistance to \(\beta\)-lactam and macrolide antibiotics found in *Streptococcus pneumoniae*\(^2\,3\), *H. aemophilus influenzae*\(^4\) and *M. oraxelle catarrhalis*\(^5\) circulating in Italy compared with that of other European countries. Recent reports pointing to a strikingly different picture in the sensitivity of *Streptococcus* pyogenes to erythromycin have therefore been met with surprise. Until 1993, reports showed local rates of erythromycin resistance in this microorganism at a 5–8% level. Starting in 1995, a large number of reports warned that the situation might have worsened rapidly. Scopetti et al.,\(^6\) analysing 596 S. pyogenes from throat swabs of patients with pharyngo-tonsillitis and scarlet fever, found macrolide resistance to exceed 15% and to be distributed in several T serotypes. A similar figure (15.5%) was reported by Millesimo & Savoia\(^7\) working on 100 unselected strains. Cipriani et al.\(^8\) obtained nationwide data on 2739 isolates from 52 Italian laboratories and found a mean level of erythromycin resistance of 24.7%. More recently, Cristiano et al.\(^9\) observed that resistance to erythromycin rose from 2.8% in 1993 to 35.3% in 1995 in over 1100 strains isolated from pharyngeal swabs taken in their region. While it must be conceded that all these studies suffer some limitations arising from their methodological heterogeneity and lack of quantitative details (disc diffusion was the only technique employed to assess susceptibility), none the less they are consistent in indicating that the in-vitro activity of erythromycin against pharyngeal isolates of *S. pyogenes* has changed at least in some areas of this country.

An abrupt emergence of resistance to this drug has been described previously in other parts of the world. Japan's experience in the 1970s is well known\(^10\) and indicated that acquisition of resistance might be linked to the levels of consumption of the drug, an observation supported by more recent findings in Finland,\(^11\) Taiwan\(^12\) and Italy.\(^9\) While erythromycin resistance has all but disappeared in Japan following restricted use of the antibiotic,\(^13\) this fact should not give rise to complacency, since the monoclonal (T 12 serotype) character of that outbreak might have been responsible for this success. Such measures are difficult to enforce and may not produce similar, favourable, results in the present situation, dominated as it is by patterns of diffuse polyclonal resistance.\(^9,12,14\)

Clinical microbiologists are liable to consider the resistance they observe in vitro as a good predictor of therapeutic failure in vivo. They are, therefore, concerned whenever a trend towards loss of susceptibility is detected in any major pathogen. This is certainly a sound and prudent attitude. In respect of *S. pyogenes*, while erythromycin has been shown to be unable to eradicate resistant strains in a variable, but minor, proportion of pharyngitis patients,\(^14–17\) this observation has never been validated by large, well-controlled studies. More thorough investigations are now badly needed, since new data emerging from the analysis of the incidence of different erythromycin resistance phenotypes have demonstrated that high-level (MIC > 64 mg/L)
constitutive macrolide, lincomamide and streptogramin B resistance is very rare (2%). The remaining strains can be categorized into two other phenotypes expressing only low-level resistance to erythromycin, with MICs of 1–16 μg/mL. One of these phenotypes, which comprises up to 60% of the total, is represented by the conventional inducibly resistant strains in which incubation with sub-inhibitory concentrations of erythromycin elicits high-level resistance to clindamycin. The third and most recently described phenotype (38–75% of the total), is characterized by low-level resistance to erythromycin, persistent susceptibility to clindamycin despite induction, and retained sensitivity to most other antibiotics. Irrespective of the phenotype, all erythromycin-resistant isolates are also resistant to other 14- and 15-membered macrolides. One interesting point reported by the Finnish authors is that, at each site investigated, only one S. pyogenes phenotype predominated.

While it may be reasonable to assume that constitutive resistance will blunt the in-vivo efficacy of a macrolide, the response to be expected from the other phenotypes (the majority) is open to speculation. This is particularly so in view of the high tissue concentrations achieved by the new macrolides as a consequence of their improved pharmacokinetics. This may overcome the low-level resistance presented by these strains and, at the same time, avoid the sub-MIC concentrations that might otherwise induce resistance in a surviving fraction of the population. Since the local prevalence of the three erythromycin phenotypes is presently unknown but might determine the outcome of treatment, it seems necessary to assess whether acquisition of any mechanism of resistance by S. pyogenes is immediately translated into a breakpoint for therapeutic failure, or if there are quantitative gradients that establish clinical relevance. Clarification of this point is of particular importance in developing guidelines for the treatment of S. pyogenes pharyngitis in patients who are allergic to β-lactams, or who have failed a course of therapy with these or other drugs and therefore risk complications and/or recurrences. The importance of the level of antibiotic resistance of respiratory pathogens in determining the outcome of therapy with drugs showing less than optimal in-vitro activity is epitomized by the ability of penicillin to overcome penicillin resistance in pneumonia caused by S. pneumoniae strains with penicillin MICs of <2 μg/mL. A relation between eradication of an isolate and its specific mechanism of erythromycin resistance may also explain why clindamycin, a drug previously not recommended for treatment of macrolide-resistant S. pyogenes, was successfully used during an outbreak, since we have now learned that strains resistant to erythromycin and other 14- and 15-membered macrolides are susceptible to clindamycin if they belong to the newly described M phenotype. Given the high prevalence of this phenotype among erythromycin-resistant S. pyogenes it might be speculated that the Swedish outbreak was caused by an M clone that could be eradicated by clindamycin therapy. Despite country-to-country variations, macrolide treatment of pharyngitis represents a minor proportion of the overall use of this class of antibiotics. The broad coverage offered against all major conventional and intracellular pathogens responsible for community-acquired lower respiratory tract infections is the main reason for widespread macrolide usage. Traditionally, macrolides also represent the preferred therapy used by dentists. Perhaps less recognized, but carrying even more far-reaching consequences, is their recent use in large, novel therapeutic indications. These include eradication of Helicobacter pylori in patients with peptic ulcer and, in patients with the acquired immunodeficiency syndrome, attempts to eradicate parasites and mycobacteria. For most of these indications optimal dosage schedules and duration of administration are not yet clarified. The huge selective pressure building up in the mass of patients treated with macrolides for all these diverse indications might explain the emergence of resistance in microorganisms both related and unrelated to the infections being treated.

So, what is the role of macrolides in acute pharyngitis caused by S. pyogenes? The resurgence of rheumatic fever in some areas of the world and the increasing incidence of invasive infections caused by group A streptococci dictate that a penicillin or a cephalosporin, drugs that have retained universal in-vitro activity against these pathogens, be employed as a first-line agent in the therapy of this condition. Despite clear indications that in-vitro resistance might be rising in some locations, treatment failures or a rise in the rate of complications from macrolide use are not being reported. This may be a consequence of the fact that pharyngitis is a minor, self-limited condition and that assessment of failure requires culturing and typing at the end of therapy, procedures rarely performed nowadays. On the other hand, this situation could also be ascribed to unhindered efficacy of the modern macrolides against S. pyogenes strains with mechanisms of resistance that are insufficient to produce clinical significance. Which of these two alternatives is correct will only be determined after the completion of further studies. In the meantime, clinical microbiologists are best advised to keep close track of the incidence of resistance in local S. pyogenes isolates. In so doing the recent dramatic changes in the breakpoints for susceptibility to macrolides introduced by the NCCLS guidelines will have to be taken into account, especially when comparing rates of resistance before and after their introduction. Determination of erythromycin resistance phenotypes also seems mandatory now. Should the constitutive strains, reportedly rare at present, become prevalent, their high-level resistance may justify clinicians avoiding the use of macrolides and lincomamides. At that stage, at least among patients allergic to β-lactams, yet another important Gram-positive pathogen will have created a therapeutic problem that is difficult to solve.
References


