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Conference summary

Summary of the international consensus symposium on management of HIV, CMV and hepatitis virus infections

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1. Introduction

Many similarities exist between our attempts to treat infections with human immunodeficiency virus (HIV), cytomegalovirus (CMV) and hepatitis B and C viruses, while patients suffering from these infections also exhibit considerable overlap. Successes in the treatment of these infections have been limited by a lack of effective agents, incomplete suppression of viral replication, the development of drug resistance, and dose-limiting drug toxicities. While the development of novel potent antiviral agents is aggressively pursued, combined antiviral treatment strategies are gaining increasing attention from the scientific community, and especially for HIV infection, have proven its benefit.

Beside the issue of how to treat these infections, important questions in the management of HIV, CMV and hepatitis virus infections are when to treat and, especially in HIV and hepatitis virus infections, how to monitor the efficacy of treatment. The clinical outcome, which includes the occurrence of opportunistic disease or death in HIV infection, and development of cirrhosis, hepatocellular carcinoma, or death in hepatitis B and C virus infections, clearly cannot be used for monitoring treatment efficacy in individual patient management. For this reason, surrogate

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markers of disease, e.g. the CD4+ lymphocyte count in HIV infection, and the transaminase levels and liver histology in viral hepatitis, have generally been used for the timing of treatment and the evaluation of treatment efficacy in the individual patient. In recent years, with the development of convenient molecular assays for quantitation of viral nucleic acids, the use of viral load in guiding treatment has gained increasing attention.

In view of the overlap with regard to current issues in the management of HIV, CMV, and chronic viral hepatitis, a consensus symposium was organized and convened by The Macrae Group (New York, NY, USA) in New York on March 14–16, 1997, which brought together leading clinical and laboratory researchers and representatives from government and industry to exchange ideas about the management of these viral infections and to discuss the future directives. At this symposium, the current status of antiviral treatment was reviewed, and the role of virus load and surrogate markers to guide treatment was discussed. This article provides a summary of the information presented.

Members of the consensus panel were C.A.B. Boucher, Utrecht University, The Netherlands; S.A. Danner, University of Amsterdam, The Netherlands; B. Gazzard (co-chair), Chelsea and Westminster Hospital, UK; P.D. Griffiths, Royal Free Hospital School of Medicine, UK; C. Katlama (co-chair), Hôpital Pitié Salpêtrière, France; and S. Vella, Istituto Superiore di Sanita, Italy. The presenters at the symposium were D.D. Ho, Aaron Diamonds AIDS Research Center, USA; P.D. Griffiths, Royal Free Hospital School of Medicine, UK; G. Dusheiko, Royal Free Hospital, UK; M. Nowak, University of Oxford, UK; B. Gazzard, Chelsea and Westminster Hospital, UK; S. Vella, Istituto Superiore di Sanita, Italy; C. Loveday, Royal Free Hospital, UK; S. Pham, Glaxo Wellcome, USA; J. Toole, Gilead Sciences, USA; B. Autran, Hôpital Pitié Salpêtrière, France; C.A.B. Boucher, Utrecht University, The Netherlands; F. Gotch, Chelsea and Westminster Hospital, UK; R. Pauwels, Institute for Antiviral Research, Belgium; R. Harrigan, Glaxo Wellcome, UK; S. Kravcik, Ottawa General Hospital, Canada; A. Japour, Abbott Laboratories, USA; R. Williams, University College London, UK; J. Chancellor, Glaxo Wellcome, UK; C. Katlama, Hôpital Pitié Salpêtrière, France; M. Markowitz, Aaron Diamonds AIDS Research Center, USA; and T. Poynard, Hôpital Pitié Salpêtrière, France.

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2. Management of HIV infection

2.1. Viral load and pathogenesis

Measurements of viral load in HIV infection were initially restricted to quantitation of HIV-1 p24 antigenaemia, and quantitative cultures of HIV-1 in plasma or peripheral blood mononuclear cells (PBMC). Using these methods, it was shown in the early 90s that primary infection with HIV is characterized by a burst of viraemia, which, concurrent with the onset of the immune response, is followed by a sharp decline of culturable virus to low or undetectable levels after 8–12 weeks (Clark et al., 1991; Daar et al., 1991). Based on these findings, it was assumed that the clinically latent phase that follows primary infection is synonymous with virological latency. However, the development of highly sensitive molecular amplification techniques for the detection of HIV-1 nucleic acid sequences have made it possible to detect virus in the peripheral blood compartment at all stages of the infection in virtually all HIV-1 infected persons. Using these techniques, high levels of HIV-1 RNA in plasma and expression of HIV-1 messenger RNA (mRNA), both indicative of active viral replication, could be measured during all stages of infection, indicating that virological latency does not exist (Schnittman et al., 1991; Michael et al., 1992; Piatak et al., 1993). Indeed, more recent evidence has demonstrated that HIV-1 infection is a highly

dynamic process with continuous high rates of viral replication throughout the course of infection, paralleled by an increased turnover of CD4+ lymphocytes (Ho et al., 1995; Wei et al., 1995; Perelson et al., 1996). Illustrating the highly dynamic nature of HIV-1 infection, it is estimated that, on average, 10×10^9 virions are cleared and produced every day, and that the half-lives of virions and productively infected cells are approximately 6 h and 2 days respectively, which, as Dr. David Ho pointed out during the symposium, are probably even overestimations of the actual half-lives with a more accurate figure of the viral half-life being as low as 30 min (Perelson et al., 1996). Histopathological examination has shown that lymphoid tissues serve as the primary site of this dynamic process and are the major reservoirs of HIV (Fox et al., 1991; Pantaleo et al., 1991; Embretson et al., 1993; Pantaleo et al., 1993).

The level of circulating virus thus reflects a dynamic equilibrium between clearance and production of virus. Although the exact mechanism of the ultimate loss of CD4+ lymphocytes is still unclear, it is plausible that the decline in CD4+ cell count is driven by these continuous rounds of viral replication in host cells with associated rapid cell turnover.

Recent studies have demonstrated that the steady-state level of circulating virus, as measured by plasma HIV-1 RNA levels, is established within 3–6 months after seroconversion, and is highly predictive of the subsequent course of disease (Mellors et al., 1995, 1996). In the light of these findings, it does not seem surprising that several studies have shown a correlation between the magnitude and duration of treatment-induced declines in HIV-1 RNA load and improved clinical outcome, although discrepancies do exist (see below) (Yerly et al., 1995; O'Brien et al., 1996; Schooley et al., 1996; Katzenstein et al., 1996). Several antiretroviral combination regimens, especially those involving three drugs, have shown to result in sustained reductions of HIV-1 RNA load to below the detection limits of currently available quantitative assays (500–1000 RNA copies/Ml.), which has led to cautious optimism that viral resistance to drugs may be prevented and that even eradication of infection might be possible. However, the presence and origin of any residual virus during treatment, particularly in protected sites, remains an important issue.

The decline in plasma virus load during potent antiretroviral treatment follows a biphasic pattern (Perelson et al., 1997). The first phase is characterized by a decline of virus load to low levels within 2 weeks, and is reflective of the rapid elimination of free virus and decay of productively infected T cells (Ho et al., 1995; Wei et al., 1995; Perelson et al., 1996, 1997). Considering an initial decline in virus load of $2 \log_{10}$ observed during potent antiretroviral regimens, it can be deduced that approximately 99% of the amount of circulating virus originates from productively infected T cells. During the second phase, the level of circulating virus more gradually declines to below detectable levels. This slower phase is thought to reflect the decay rate of sources of HIV-1 other than activated T cells, such as long-lived infected cell populations, e.g. tissue macrophages or dendritic cells, or latently infected lymphocytes which produce virus upon activation (Perelson et al., 1997). Detailed mathematical analysis has indicated that the loss of long-lived infected cell populations, mainly tissue macrophages, is a major contributor to the second phase of decay, while activation of latently infected cells only seems a minor source (Perelson et al., 1997). In accordance with this analysis, it has been found that the pool of latently infected resting T cells with replication-competent integrated provirus is low ($< 10^7$ cells) (Chun et al., 1997). It has been estimated that, at the pretreatment steady state, the proportion of plasma virions produced by long-lived infected cells ranges from 1 to 7%, compared to $< 1\%$ produced by activation of latently infected lymphocytes (Perelson et al., 1997). The remaining 93–99% of plasma viraemia originates from productively infected T cells.

Based on the duration of the second phase of RNA decay, it has been calculated that complete eradication of the virus would require 0.5 to 3 years of fully suppressive treatment, depending on the poolsize of long-lived infected cells (10^8–10^{12} cells) and the half-life of these cells (1–4 weeks) (Perelson et al., 1997). However, while the concept of eradication of HIV is challenging, several

questions still need to be answered. Although latently infected CD4+ lymphocytes are present at low frequency, the majority of which seems to be harbouring unintegrated or defective proviral DNA (Chun et al., 1997), this small reservoir still warrants consideration since these cells may persist for many years, leaving open the possibility of reignition of viral replication. These cells or other small undetected compartments may account for a yet undetected slow third-phase decay of plasma viraemia, below the detection limit of currently available quantitative assays. In addition, it remains to be studied whether sustained complete suppression of viral replication occurs in lymphoid tissues, where most of the virus is produced, and other solid tissues. While impressive decreases in viral burden have been demonstrated in lymphoid tissues during three-drug treatment with protease- and reverse transcriptase inhibitors, virus-producing mononuclear cells could still be detected after 6 months of therapy, albeit at low numbers (Cavert et al., 1997). The life-span and nature of these cells require further research. The presence of sanctuary sites of viral replication, e.g. the central nervous system, must be considered, which may not be amenable to inhibition by some or all of the antiretroviral drugs, for example due to pharmacokinetic limitations. Even when triple drug combination treatment is instituted, not all three drugs may be equally effective in such sanctuaries, resulting in local suboptimal suppression of viral replication and increased risk of resistance development.

Future research should be directed to addressing these issues, for the purpose of which highly sensitive assays for measuring virus load, including HIV-1 RNA in plasma and proviral DNA in tissues and cells, are clearly needed. Moreover, to investigate the function and potential importance of any residual viral genomes, the development of sensitive infectivity assays as well as sensitive assays to monitor mRNA expression in tissues, is warranted. Ideally, evidence of viral replication should be monitored in all potential sanctuaries of viral replication, but would require easy access to these sites.

2.2. Immune reconstitution

With the widespread use of potent combination treatment regimens, the clinical benefits of these regimens are becoming increasingly clear, as evidenced by declining hospitalization and mortality rates and reports of resolving opportunistic infections and maligancies, such as protozoal diarrhoea, oral candidiasis, progressive multifocal leukoencephalopathy and Kaposi's sarcoma (Zingman, 1996; Foudraine et al., 1997; Murphy et al., 1997; Power et al., 1997). This indicates that potent and sustained suppression of viral replication, at least to a certain extent, is associated with reconstitution of the immune system. However, the question remains whether complete immune reconstitution is attainable with potent antiretroviral therapy. This not only includes repopulation of the lymphoid organs, but also restoration of the immune function and T cell repertoire.

Dr Brigitte Autran (Hôpital Pitié Salpêtrière) gave an excellent review of her studies investigating immune reconstitution during antiviral therapy (Autran et al., 1997). Preliminary results of a 6 month study in 188 advanced pretreated HIV-1 infected patients who were treated with either ritonavir or indinavir showed that the initial CD4+ cell count does not seem important in determining CD4+ cell recovery, as suggested by a similarity in absolute CD4+ cell rises, irrespective of the baseline CD4+ cell count. Furthermore, while decreases in virus load were clearly shown to be pivotal for immune reconstitution, clearance of viraemia alone did not seem sufficient, indicating that other mechanisms also play a role. A subgroup of patients only showed a minor increase in CD4+ cell count after 6 months of therapy (median 45 cells/mm^3), despite a greater than 2 $\log_{10}$ decrease in plasma HIV-1 RNA load. Interestingly, rather than an immunological failure, this subgroup seemed to exhibit a delayed response, since, in contrast to other subjects, the observed CD4+ cell increase in this group occurred only beyond two months of treatment. This suggests that differences in homeostatic mechanisms may account for some of the differences in CD4+ cell recovery between patients.

In a 12 month study of antiretroviral-naive patients (baseline CD4+ cell count 165 cells/mm^3) who were treated with a three-drug combination of zidovudine, zalcitabine and ritonavir, the long-term kinetics of CD4+ cell recovery were analyzed in detail. In this group of patients, a rapid increase in CD4+ cells was observed within the first month, followed by a more gradual rise during subsequent months, achieving an absolute increase of 200 cells/mm^3 after 12 months of treatment. Analysis of memory and naive CD4+ cell subpopulations during treatment, showed that, by far the largest proportion of the CD4+ cell gain consists of memory T cells. However, while the percentage of naive T cell subpopulations did not change during the first 4 months of treatment, a significant increase in the proportion of these cells was observed during the ensuing 8 months. This suggests that thymic or extra-thymic sources to a certain extent do have the capacity to repopulate the pool of naive T cells, which may contribute to immune reconstitution.

In the same study, the effect of treatment on the activation state of CD4+ and CD8+ lymphocytes was evaluated, and showed that, parallel to the drop in virus load, the proportion of activated CD4+ and CD8+ lymphocytes decreased significantly. This suggests that either the virus itself or the antigenic pressure of the virus is responsible for the abnormally high activation state of T lymphocytes, characteristic of HIV-1 infection. Decreasing the virus load thus results in a loss of abnormal activation of T lymphocytes, and probably their sensitivity to apoptosis. Concurrent with the decrease in activated lymphocytes, an increase in the number of T cells expressing the CD28 molecule was observed. This suggests that some restoration of T cell function occurs, since the CD28 molecule has an important function in allowing CD4+ lymphocytes to activate in response to antigen and in protecting them from apoptosis.

Indeed, since a loss of T cell function heralds the progressive immunodeficiency during HIV-1 infection, an important question is whether repopulation of CD4+ lymphocytes during antiretroviral treatment is associated with improved T cell function. In a small study presented by Dr. Autran, the in vitro proliferative responsiveness of T cells to specific antigen (tuberculin or CMV proteins) was evaluated in 15 patients treated with either ritonavir or indinavir. In contrast to a previous study in patients treated with ritonavir (Kelleher et al., 1996), it was shown that immune responsiveness could even be restored in patients completely lacking a proliferative T cell response prior to treatment. The current study showed that the proportion of patients capable of eliciting antigen-specific proliferative responses increased from 20% at baseline to 66% within 12 weeks of treatment. In a further analysis, it was shown that, compared to patients without evidence of improved immune function, the patients with improved antigen-specific T cell responsiveness also exhibited significantly higher increases in absolute CD4+ cell counts, as well as in the naive, memory, and CD28+ subpopulations. While the decreases in virus load were also more pronounced in the latter patient group, these differences were not statistically significant. Interestingly, the improvement in T cell responsiveness to tuberculin or CMV proteins was already observed after 2–4 weeks of treatment, at a time when the increase in CD4+ cells only consisted of memory T cells, and naive cells were not yet repopulated. This indicates that repopulation of naive cells is not strictly necessary for recovery of immune responsiveness to antigens that have already been encountered in the past, but that, to a certain extent, repopulation of only memory cells suffices, presumably because low but undetectable numbers of specific memory cells persist.

Finally, in addition to an absolute loss of CD4+ lymphocytes and T cell function, the immunodeficiency of HIV-1 infection is also characterised by a progressive loss of the diversity of the T cell repertoire. While further research is ongoing, preliminary studies suggest that potent antiretroviral therapy may also restore the T cell Vβ repertoire to some extent.

In conclusion, even in pretreated patients with very low CD4+ cell counts and absent immune responsiveness, potent antiretroviral treatment is, at least in part, capable of restoring the immune system, including repopulation of both memory

and naive T cell subpopulations CD4+ and CD28+ lymphocytes, loss of abnormal T cell activation, improvement of antigen-specific T cell responses, and broadening of Vβ repertoires, all of which should offer protection to opportunistic infections to some degree. However, the question remains whether complete immune reconstitution is possible. While substantial increases in CD4+ cell count and functional improvement are observed in patients receiving three-drug combination treatment, normal values are generally not attained, despite sustained decreases of plasma viraemia to below detectable levels. Is this due to ongoing viral replication or to an inability to completely reconstitute the immune system, e.g. because of irreparable damage, or does complete immune reconstitution just require extended periods of time? Further research is clearly required to explain the differences in the extent of immune recovery among individuals, and to assess whether prolonged suppression of plasma viraemia will ultimately result in complete immune reconstitution. Furthermore, it needs to be investigated whether complete immune competence is maintained when antiretroviral treatment is started early in the course of infection, e.g. before a loss of T cell repertoire has occurred.

2.3. Management and treatment

In recent years, considerable progress has been made in the treatment of HIV-1 infection. Several potent antiretroviral agents, including reverse transcriptase- and HIV protease-inhibitors, are now readily available, while the development of novel antiviral agents continues. Prolonged reductions of plasma viraemia to undetectable levels have been observed during several antiretroviral regimens, especially those involving combinations of three drugs, which allows for cautious optimism in the management of HIV-1 infection.

In the care of individual patients, important questions are when to initiate treatment and when to change it. Obviously, evaluation of treatment efficacy in the individual HIV-infected patient cannot depend on the clinical outcome, i.e. development of opportunistic diseases or death, which necessitates the use of surrogate markers of disease. The number of CD4+ lymphocytes is predictive of the clinical outcome, and, in the absence of other useful markers, has long been used as the most important surrogate marker of disease. However, treatment-induced changes in the CD4+ cell count have clearly been shown to be unreliable predictors of the long-term clinical treatment effects (Choi et al., 1993; O'Brien et al., 1996). The development of readily available assays for measurement of plasma HIV-1 RNA has ignited increasing enthusiasm for the use of plasma viraemia in monitoring treatment efficacy and determining the optimal time to start treatment. In part, this enthusiasm is inspired by the notion that plasma viraemia directly reflects the level of viral replication which seems obviously relevant in the pathogenesis of this viral disease. In addition, increasing evidence suggests that the level of plasma virus load is closely correlated with the level of virus load in lymphoid tissues, where most of the viral replication occurs (Cavert et al., 1997; Chun et al., 1997; Perelson et al., 1997). As mentioned previously, the plasma HIV-1 RNA level is highly predictive of the clinical outcome. A single measurement of plasma HIV-1 RNA yields more accurate prognostic information than a single CD4+ cell count, especially at CD4+ cell counts higher than 500 cells/mm^3 (Mellors et al., 1995, 1996).

Measuring plasma viraemia is clearly useful for monitoring treatment efficacy, at least in the short term. Most importantly, there is a clear association between resurgences in virus load, indicative of treatment failure, and the appearance of drug-resistant virus variants (Schuurman et al., 1995; Wei et al., 1995; De Jong et al., 1996). It seems obvious that, in the long term, sustained declines in plasma viraemia are associated with improved clinical outcome. Indeed, several studies have shown a correlation between the magnitude and duration of treatment-induced declines in plasma HIV-1 RNA load and a decreased risk of progression or death (Yerly et al., 1995; Katzenstein et al., 1996; O'Brien et al., 1996; Schooley et al., 1996). However, a number of large-scale randomized studies have also shown discrepancies in the relation between suppression of HIV-1 RNA levels and the clinical outcome. For example, in the

ACTG 175 study, there were no differences in the risk of progression between patients treated with didanosine alone and patients receiving combined zidovudine/didanosine treatment, while suppression of plasma HIV-1 RNA levels clearly was superior in the latter group (Katzenstein et al., 1996). In addition, the clinical outcome was better in patients treated with the zidovudine/didanosine combination than in patients receiving zidovudine and zalcitabine, while decreases in plasma virus load were similar in both groups (Katzenstein et al., 1996). These discrepancies indicate that the effect of treatment on plasma viraemia may not be completely proportional to the clinical benefit. Additional factors that may influence the beneficial clinical effects of treatment, and may not be captured by the changes in plasma viraemia, include drug-related factors, e.g. toxicity, and factors related to the host, e.g. the immune system, or to the virus, e.g. the syncytium-inducing capacity of the virus or the amount of residual virus in the central nervous system or in visceral reservoirs.

Nevertheless, it seems safe to state that the goal of antiretroviral treatment should be to keep the level of plasma HIV-1 RNA as low as possible for as long as possible. While the ultimate objective of treatment is to reduce the plasma HIV-1 RNA load to zero, the sensitivity of currently available quantitative assays is limited. The current tendency in clinical practice, expressed by several opinion leaders, is to aim for reducing plasma virus load to undetectable levels. However, one should bear in mind that this is a rather arbitrarily chosen measure for 'as low as possible'. The detection limit varies between quantitative assays, and little is known about the amount and relevance of any residual virus below the limit of detection. The concept of eradication set aside, it may even be that a reduction to a certain threshold level of detectable virus would yield similar beneficial effects as a reduction to below the limits of currently available quantitation assays. Furthermore, it is not clear whether suppression of viraemia to undetectable levels is achievable in all patients. While clinical studies suggest that this is achievable in most, if not all, previously untreated patients, it seems doubtful that this is the case in pretreated individuals. In addition, long-term follow-up of clinical studies is needed to assess whether suppression of plasma viraemia can be maintained.

The definition of a treatment-failure is clearly important in making treatment decisions based on virus load. If the aim of treatment is to reduce virus load to undetectable levels, should the mere detection of virus, irrespective of the amount, be defined as a treatment failure or would a certain threshold level of virus load be acceptable? A resurgence of virus load to baseline levels is clearly indicative of a treatment-failure, and should always lead to reconsideration of the antiretroviral treatment regimen, if possible based on drug-resistance testing. Evaluation of drug resistance should ideally include both phenotypic and genotypic analysis, since knowledge on genotypic resistance patterns is predominantly based on monotherapy, and alternative, yet unknown mutational pathways may occur during combined antiviral therapy. Furthermore, a detailed drug history is mandatory for a rational choice of alternative drugs. It should be considered that, while evidence of drug resistance is lacking in circulating virus, drug resistant viruses may persist in cells in individuals previously, but not currently exposed to a particular drug.

It can be concluded that, while sustained suppression of virus load to as low as possible levels seems desirable, controlled clinical studies are needed to evaluate the optimal use of plasma virus load and drug resistance measurements in monitoring treatment efficacy and making treatment decisions. Studies attempting to address some of the above discussed issues are in progress.

The optimal time to initiate treatment is another issue which requires further study. Several researchers have advocated early and aggressive treatment, as incarnated in the paradigm 'hit hard and hit early', aiming at prevention of the development of drug resistance (Ho, 1995; Lange, 1995). This paradigm was proposed when it became clear that, throughout the course of infection, HIV-1 infection is characterized by persistent high levels of viral replication, which, coupled with the high mutability of the virus, allows for the continuous generation of genetic

virus variants, including viruses that are drug resistant (Coffin, 1995).

The 'hitting hard' component is aimed, on one hand, at suppressing all resistant viruses that are present before treatment by instituting a combination of potent drugs with non-overlapping resistance patterns, and on the other hand, to suppress viral replication to such a degree that novel resistant variants do not develop. The idea behind early intervention ('hitting early') is to prevent the development of drug resistance by starting suppressive treatment at a time when the number of elapsed viral generations, and thus the number of pre-existing resistant virus variants, is still limited. An additional, maybe more important rationale for early intervention is that, early in the course of infection, the immune system is still relatively intact. As discussed before, it remains to be seen whether, even with the current potent antiretroviral combination regimens, complete immune reconstitution, including T-cell function and repertoire, is achievable in severely immunocompromised patients.

While there is little doubt about the merits of aggressive suppressive treatment and the general consensus is not to delay intervention until severe immunodeficiency or AIDS has developed, the optimal timepoint to initiate treatment is still under debate. Studies evaluating the efficacy of combined therapy with protease- and reverse transcriptase inhibitors instituted early after seroconversion are ongoing. As presented by Dr. Martin Markowitz (Aaron Diamonds Center for HIV Research), preliminary results of these studies are promising, showing disappearance of detectable and culturable virus from plasma, lymphoid tissues and semen in virtually all patients who are compliant to therapy. However, declines of virus load in plasma and lymphoid tissues to below detectable levels have also been observed in patients receiving triple drug combinations at later stages of the infection. While the long-term follow-up of both strategies needs to be awaited, this may suggest that 'hitting hard' is more important than 'hitting (very) early'.

Until proven otherwise, treatment of HIV-1 infection requires the chronic administration of a combination of potent drugs for an indeterminate, possibly life-long period of time. A disadvantage of early treatment is the prolonged exposure to the considerable toxicities associated with potent antiretroviral combination regimens, which, even in the short term, warrant therapy withdrawal in a substantial proportion of patients. The long-term toxicities of current regimens are still unknown. Besides toxicity, compliance issues also need to be considered, as non-compliance to therapy carries the grave consequence of generating (multi-)drug resistant virus variants. Current combination regimens imply the administration of a large number of tablets or capsules at several well-defined timepoints during the day, which demands a high degree of discipline, especially in HIV-infected individuals who feel healthy otherwise. Besides the risk of non-compliance, the effects of toxicities and the strict schedules of drug-administration on the quality of life also warrants consideration. Besides patient-related issues, pharmaco-economic analyses of cost-effectiveness also need to be taken into consideration in determining the optimal time for intervention.

It should be remembered that the natural course of infection exhibits a large degree of variability. Therefore, the concept of eradication set aside, blindly starting treatment early in the course of infection may thus not equally benefit all patients. For example, while rapid progressors would probably benefit from treatment, long term non-progressors would possibly be unjustly exposed to the toxic effects of treatment for a prolonged period of time. Ideally, the optimal timepoint to initiate treatment should be based on one or more predictive markers, such as the CD4+ cell count or plasma HIV-1 RNA load. While, as discussed above, the latter is highly predictive of the clinical outcome, the value of a single measurement of plasma HIV-1 RNA load in deciding when to initiate treatment in the individual patient is unclear.

In conclusion, until more knowledge on the rational use of markers becomes available, the decision to initiate treatment should probably be guided by pragmatism, i.e. start treatment at a time when the immune system is relatively intact and the risk of opportunistic disease is relatively low (e.g. plasma virus load >10.000 HIV-1 RNA

copies/ml and/or a CD4+ cell count $<350-500$ cells/mm^3). In view of the high risk of resistance development, initial treatment should be aggressive, involving three drugs with non-overlapping resistance patterns, including inhibitors of HIV protease and reverse transcriptase. In case of treatment-failure, by whatever definition, the choice of substitutive therapy should ideally be based on resistance-testing and drug histories, to ensure optimal suppression of viral replication by the new regimen. For similar reasons, if toxicity warrants withdrawal of one drug in a combination regimen, it is strongly advised to temporarily discontinue all drugs of the combination, and reinsitute a modified potent combination when the toxicity has resolved.

3. Management of CMV infection

Human cytomegalovirus (CMV) rarely causes disease in immunocompetent persons, but represents a major pathogen in immunocompromised patients, most notably transplant recipients and HIV-infected individuals. The major disease manifestations are retinitis in HIV-infected persons, and pneumonitis in bone marrow and solid organ transplant patients. Other CMV diseases include gastrointestinal manifestations, hepatitis, encephalitis, and disseminated CMV infection. In HIV-1 infection, the development of CMV disease is clearly correlated with the severity of immunodeficiency, occurring predominantly in patients with CD4+ cell counts lower than 50 cells/mm^3 (Kuppermann et al., 1993). Rather than primary infection, CMV disease in the immunocompromised usually results from reactivation of latent infection which fails to be controlled by the diminished cell-mediated immune response. In the case of recipients of solid organ transplants, reactivation frequently occurs in the donated solid organ if the donor is seropositive.

Currently available treatment options for CMV disease include ganciclovir, foscarnet, or cidofovir. These drugs seem to be equally effective in controlling retinitis in AIDS patients. However, despite chronic maintenance therapy with these drugs, relapses of disease almost invariably occur. This is most likely to be due to poor penetration of drugs to the eye. While this problem is circumvented by intravitreal administration of the drug, this does not give systemic protection against CMV. The development of drug resistance during prolonged suppressive treatment may also be important in drug failure. As is the case for other viruses, most notably HIV, incomplete suppression of viral replication during chronic antiviral treatment predisposes to the development of drug resistance. Indeed, the emergence of CMV strains with reduced susceptibility to drugs during chronic maintenance therapy is well documented, and has been associated with increases in plasma CMV load and treatment failure (Boivin et al., 1996). Reduced susceptibility to ganciclovir is conferred by specific mutations in the UL97 gene, which encodes for the viral phosphotransferase responsible for the initial phosphorylation of ganciclovir, as well as in the viral DNA polymerase gene (UL54) (Chou et al., 1995; Baldanti et al., 1996; Smith et al., 1997). Since foscarnet does not require phosphorylation for its activity, and cidofovir is already monophosphorylated, resistance to these drugs is conferred by mutations in the viral DNA polymerase gene only. Dual resistance to these drugs has also been documented (Knox et al., 1991; Sarasini et al., 1995; Baldanti et al., 1996). High level, but not low level, ganciclovir-resistant virus variants exhibit reduced susceptibility to cidofovir, suggesting that mutations in the polymerase gene confer higher levels of ganciclovir resistance and cross-resistance to cidofovir (Smith et al., 1997).

Treatment regimens with improved antiviral potency are clearly needed. Ganciclovir and foscarnet act synergistically in vitro, and combined therapy with these drugs has been shown to prolong the progression-free interval in patients with relapsed retinitis when compared to monotherapy with either drug alone (Studies of Ocular Complications of AIDS Research Group, 1996). However, visual acuity outcomes in patients receiving combined treatment were similar to patients treated with monotherapy in the intention to treat analysis, while the quality of life in the former group of patients was significantly diminished due to longer infusion times and increased toxicity

(Studies of Ocular Complications of AIDS Research Group, 1996). A number of novel agents are currently in clinical development and may prove to be valuable additions to the treatment arsenal, including lobucavir, bispom-PMEA, and a benzimidazole (1263GW), as well as a number of antisense oligonucleotides.

While improved suppression of viral replication will undoubtly have beneficial effects, antiviral treatment does not necessarily reverse secondary pathological processes which may have been triggered by CMV. For example, the lung damage of CMV pneumonitis in bone marrow transplant recipients seems to result from the cell-mediated immune response provoked by CMV, rather than from the direct effects of the virus itself (Grundy et al., 1987). This may explain why established CMV pneumonitis in these patients generally responds poorly to anti-CMV treatment.

In general, anti-CMV treatment is most effective when instituted early in the course of disease. In the case of CMV retinitis, it is imperative to diagnose CMV infection early, when lesions are restricted to the periphery, in order to prevent the loss of visual acuity associated with progression of disease to central regions of the retina. For this reason, it is recommended in current clinical care that all CMV-seropositive HIV-infected patients with CD4+ cell counts less than 100 cells/mm^3 should have 3–4 monthly ophthalmological examinations, and high-dose induction therapy, followed by chronic maintenance dosing, should be instituted in case of evident retinitis (Dunn and Jabs, 1995).

Alternatively, prevention of CMV disease by prophylactically treating all CMV-seropositive, severely immunocompromised patients, has been advocated. Indeed, in a placebo-controlled study in HIV-infected patients with less than 100 CD4+ cells per mm^3, prophylactic treatment with oral ganciclovir reduced the risk of development of CMV disease by 50% (Spector et al., 1996). However, 74% of placebo-recipients in this study did not develop CMV disease during the study period, suggesting that prophylactic treatment was not (yet) needed in a large proportion of patients. In bone marrow transplant recipients, prophylactic treatment with ganciclovir has been shown to reduce the risk of CMV disease without a commensurate effect on the mortality, because the toxicity of the treatment (neutropenia) appears to counterbalance the beneficial effects on CMV disease (Goodrich et al., 1993; Winston et al., 1993). These observations indicate that improved identification of patients at high risk of developing CMV disease is desirable, for which purpose increasing evidence suggests an important role for measurements of CMV load.

Established CMV disease is characterized by high blood levels of CMV, indicating an important role of virus load in the pathogenesis of CMV disease (Fox et al., 1995). Several studies have shown that levels of detectable CMV in blood or urine, as detected by viral culture or molecular methods, are higher in patients with symptomatic disease than in CMV seropositive individuals who remain asymptomatic (Kidd et al., 1993; Rasmussen et al., 1995). Furthermore, in AIDS patients with retinitis, high CMV DNA load in blood, as measured by PCR, is associated with a shorter relapse-free interval during treatment and a significantly reduced survival (Bowen et al., 1996). Although CMV may not be detectable in a high proportion of patients with relapsing retinitis, it seems likely that viraemia is required for the virus to seed to the retina to produce initial infection.

Recent prospective studies have shown that the detection of CMV in blood is predictive of the development of CMV disease (Kidd et al., 1993; Rasmussen et al., 1995; Bowen et al., 1997; Dodt et al., 1997). CMV can be detected in the bloodstream by viral culture, by detection of the pp65 antigen in leucocytes, or by molecular methods. While detection of CMV by viral culture is the most commonly used method in diagnosing CMV disease, the sensitivity and predictive value for development of disease are relatively low, which limits its use in identifying high-risk patients (Salmon et al., 1990; Zurlo et al., 1993). Additional disadvantages include the requirement of fresh samples and the fact that it may take several weeks before a reliable result is obtained. Detection of pp65 antigenaemia is strongly correlated with clinical CMV disease (Revallo et al., 1989; Landry and Ferguson, 1993). Although this

method is more sensitive, the predictive value for development of disease seems similar to viral cultures. While this method also requires fresh samples, an advantage is the possibility to semiquantify CMV, which appears especially useful in monitoring the response to treatment. A more sensitive method for detection of CMV in body fluids or tissues is the detection of CMV DNA by PCR. Prospective studies in transplant recipients and severely immunodeficient HIV-1 infected persons have shown that PCR detection of CMV DNA in whole blood, plasma or serum is highly sensitive, while the predictive value for development of disease is superior to both viral culture and antigen detection (Gerna et al., 1990, 1991; Spector et al., 1992; Kidd et al., 1993; Hansen et al., 1994). In addition, quantitative analyses of CMV DNA levels have shown that high absolute levels of virus load, as well as increases of virus load over time carry the worst prognoses.

The high predictive value of CMV viraemia as detected by molecular methods supports its use in preemptive therapy for CMV infection, i.e. therapy in the absence of manifest disease, but in the presence of signs of active viral replication, which aims at preventing the development of manifest CMV disease. In transplant recipients, preemptive anti-CMV therapy has clearly shown to be beneficial in preventing CMV disease, for which purpose PCR detection of viraemia or viruria is increasingly used in the routine clinical management of these patients (Rubin, 1991; Einsele et al., 1995). Similarly, preemptive treatment with valaciclovir has shown promising effects in reducing the risk of developing retinitis in high-risk HIV-1 infected patients. Additional controlled clinical studies evaluating the efficacy of PCR-based preemptive treatment with licensed or investigational agents are in progress. These studies should include cost-benefit analyses.

Besides its use in identifying high-risk patients, quantitative molecular methods for detection of CMV viraemia can also be used to monitor the response to treatment, and may allow for individualization of therapy. In view of the apparent pathogenic importance of CMV load, decreasing virus load should clearly be a goal of treatment.

4. Management of hepatitis virus infections

4.1. Hepatitis B virus

Infection with hepatitis B virus results in chronic hepatitis in 5–10% of immunocompetent adults, and 20–40% of immunocompromised patients, and represents a major cause of death worldwide due to the development of cirrhosis and hepatocellular carcinoma. The current status of treatment has been reviewed during a separate consensus symposium on combined antiviral therapy, a summary of which was recently published in this journal (De Jong et al., 1997). Currently, the only approved agent for chronic HBV infection is interferon-alpha, which, as measured by clearance of HBV DNA, is only effective in approximately 40% of patients with chronic hepatitis, and 35% of cirrhotic patients. As interferon primarily acts by amplifying the existing immune response, patients with active hepatitis, as reflected by increased transaminase levels, detectable HBe antigen, and active liver histology, generally exhibit the most favourable responses (Brook et al., 1989). In the immunotolerant phase of infection, characterized by minimal hepatitis, the response rate is very low.

In addition to immunomodulatory treatment, which results in augmented immune lysis of infected hepatocytes, suppression of viral replication is clearly warranted in controlling HBV infection. For this purpose, the nucleoside analogue lamivudine has shown promising effects, as evidenced by suppression of plasma HBV DNA load to below detectable levels in virtually all individuals treated with this drug at dosages of 100 mg per day or higher, accompanied by clearance of HBe antigen in some patients (Benhamou et al., 1995; Dienstag et al., 1995). However, after 6 months of therapy, relapses of viral replication almost invariably occur, which are caused by the stability and persistence of the covalently closed circular intermediate of HBV DNA (cccDNA). Clearance of the pool of cccDNA probably requires more complete suppression of viral replication, for which purpose combined antiviral therapy may be warranted. The nucleoside analogue famciclovir has shown favourable reponses

in vivo and exhibits synergistic activity with lamivudine in vitro (Korba, 1995). Studies evaluating the efficacy of this nucleoside analogue combination are ongoing. An additional rationale for combined antiviral therapy is prevention of drug-resistance. Reductions in HBV drug susceptibility have been reported during monotherapy with lamivudine, and are associated with viral breakthrough during treatment (Ling et al., 1996; Tipples et al., 1996; Bartholomew et al., 1997). Similar to the mutation at position 184 of HIV-1 reverse transcriptase, HBV drug-resistance to lamivudine is conferred by a mutation in the YMDD motif of the polymerase gene, albeit at a lower frequency than is the case for HIV-1, but other mutations have been reported as well.

Besides combining antiviral drugs, studies of combined therapy with nucleoside analogues and immunomodulatory agents, such as interferon, are in progress. Theoretically, such combinations are sensible, since progression of liver disease is driven by both viral replication and immunedestruction of hepatocytes.

By analyzing the decay rate of plasma HBV DNA levels during lamivudine treatment, it has been shown that, similar to HIV-1 infection, HBV infection is a dynamic process with a half-life of free virus of only 24 h (Nowak et al., 1996). In contrast to the situation for HIV-1, the decline of HBV DNA levels during treatment reflects the decay rate of free virus, and not the decay rate of infected cells, since, rather than preventing new infections, lamivudine prevents the production of virus from infected cells in HBV infection. By analyzing the increase in virus load after cessation of therapy, the half-lives of infected cells were determined, which showed a high variation, ranging from 10 to 100 days. This high variation in cell turnover, which stands in contrast with HIV-1 infection, can be explained by the fact that, unlike HIV-1, HBV is not a cytopathic virus. The turnover of infected hepatocytes is thus solely dependent on cytotoxic T-lymphocyte-mediated immune lysis, which exhibits a large degree of variation between patients. In theory, if viral replication is completely inhibited by antiviral drugs, the half-life of infected hepatocytes will determine the duration of curative therapy. It has been calculated that, in case of a short half-life of infected cells, i.e. 10 days, eradication of HBV would require approximately 1 year of completely suppressive treatment. However, in case of chronic persistent hepatitis, characterized by minor or absent immunelysis and consequently long half-lives of infected hepatocytes, the duration of curative therapy would be extended to many years of treatment. Theoretically, this duration may be shortened by enhancement of the immune system by combining antiviral drugs with immunomodulatory agents.

In the clinical care of HBV-infected HBs antigen-positive individuals, the decision to initiate treatment requires an evaluation of disease activity and the level of viral replication, for which purpose respectively serum transaminase levels and histology of the liver, and detection of HBe antigen and serum HBV DNA levels are generally used. It follows that the aim of treatment is to prevent or improve liver damage with resulting normalization of transaminase levels, and clear HBe antigen and HBV DNA levels. As it directly reflects the level of viral replication, measurements of HBV DNA levels appear the most reliable and important tool for estimating viral activity. Several molecular methods for assessment of serum HBV DNA levels are available, including hybridization assays and the more sensitive nucleic acid-based amplification techniques, e.g. branched DNA signal amplification- and PCR assays. In addition to their potential prognostic importance for the purpose of treatment decisions, sensitive HBV DNA quantitation assays are clearly important for the monitoring of treatment efficacy.

4.2. Hepatitis C virus

Infection with hepatitis C virus (HCV) results in chronic hepatitis in approximately 70% of cases, and, similar to HBV, is an important cause of progressive liver disease. As reviewed previously in this journal, the response rate to interferon therapy is relatively low, with sustained responses observed in approximately 25% of non-cirrhotic patients, and in only 5% of patients with cirrhosis (De Jong et al., 1997). The addition of ribavirin to interferon therapy appears to substan-

tially enhance the antiviral efficacy of treatment, as evidenced in a number of small studies (Brillanti et al., 1994; Braconier et al., 1995; Brillanti et al., 1995; Chemello et al., 1995; Schvarcz et al., 1995). However, these promising effects need confirmation in larger scale and randomized clinical endpoint studies, which are currently in progress. While novel potent agents are clearly needed, progress in this field is limited by the lack of culture systems or animal models. The recently described crystal structure of the viral serine protease will facilitate structure-based design of agents directed against this potential target for antiviral therapy (Kim et al., 1996).

As the clinical outcome of HCV infection is highly variable, the selection of patients to receive treatment is difficult. In view of the very low response rate in cirrhotic patients, it is imperative to start treatment before progression of liver disease to cirrhosis occurs (Jouet et al., 1994). For this reason, regular histologic examinations of liver biopsies remain warranted. The viral genotype and HCV RNA load influence the prognosis in HCV infection as well as the response to treatment, i.e. genotype 1, heterogeneous viral quasispecies, and high viral loads are all associated with an increased risk of progressive liver disease and a poor response to treatment (Kanai et al., 1992; Yamada et al., 1992). However, in individual patient management, the use of these virological factors in deciding when to treat, and in predicting who will benefit from treatment, remains unclear. However, measurements of HCV RNA levels, for which purpose several nucleic acid–based amplification assays are readily available, are clearly useful in monitoring the response to treatment.

5. Conclusion

The increasing knowledge of the pathogenesis of HIV-1, CMV and hepatitis B and C virus infections directly influences the management of patients with these infections. The level of viral load, directly reflective of active viral replication, is clearly important in the pathogenesis of all four infections. Quantitation of viral load, for which several convenient molecular assays are now readily available, seems an important tool for guiding treatment against these infections. However, the optimal use of viral load measurements in individual patient management requires further evaluation. Further research is also needed to determine optimal treatment strategies, especially with regard to the timing of treatment. In addition, while there has been considerable progress in the field of antiviral therapy in recent years, continuing efforts should be made in identifying novel potent antiviral drugs.

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