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Hot News

Welcome to «Hot News», a section of AIDS Reviews written by the Editors which focuses on recently reported information believed to be of both impact and higher interest to the readership.

A codon T69SSS insertion causes resistance to multiple nucleoside analogues

Combination therapy eventually forces the acquisition of mutations that are distinct from those observed with monotherapy, and can have a broad resistance spectrum.

Patients who receive AZT plus ddI or AZT plus ddC, are prone to develop a mutation at RT position 151 (Q→M), which confers resistance to all nucleoside analogues. This primary mutation is usually accompanied by other compensatory ones at codons 62, 75, 77 and 116. The prevalence of this multidrug-resistant mutation is currently low, around 2-4% in subjects previously pretreated with nucleoside analogues (Schmit J-C *et al, AIDS* 1998; 12: 2005-15; Gómez-Cano M *et al. AIDS* 1998; 12: 1015-20).

At the Lake Maggiore workshop (June 1998), it was reported that the insertion of two amino acids (usually two serine) between those at positions 69 and 70 at the RT, together with an amino acid change at position 69 (T→S), could induce a critical structural change at the enzyme, allowing RT to become multiresistant to all known nucleoside analogues (Winters M *et al*. *J Clin Invest* 1998; 102: 1769-75). Although this insertion had been reported previously, its ability to induce multiresistance has only been recognized recently. Among the required conditions for the development of this insertion seems to be a previous exposure to AZT. At the recent International Conference on the Discovery and Clinical Development Antiretroviral Therapies (St. Thomas, December 1998), a Spanish team reported four additional cases of the codon 69 insertion, and concluded that the prevalence of this insertion after testing 475 individuals failing to a previous antiretroviral combination which included at least two nucleosides, was only of 0,8%.

High risk of opportunistic infections shortly after beginning HAART

Several recent reports have pointed out a higher than expected risk of AIDS illnesses during the first 3 months on potent antiretroviral therapy in subjects having low CD4+ counts at baseline. Although some events represent relapses of previous infections, such as recurrent CMV retinitis, others constitute the first AIDS-defining disease in those individuals. In their report, Michelet *et al* (AIDS 1998; 12: 1815-22) recorded 18 clinical events during the first 2 months on HAART in subjects showing both a dramatic virological and immunological response. CMV retinitis and mycobacterial diseases, either tuberculosis or MAC disseminated infection, were the most common clinical events in this population. In previous reports in The Lancet (see Jacobson *et al.* Lancet 1997; 349: 1443-5; and Race *et al.* Lancet 1998; 351: 252-5) it was noticed that acute clinical presentations of previously latent pathogens, might occur in AIDS patients early after beginning HAART. During the 4th International Congress on Drug Therapy in HIV Infection (Glasgow, November 1998), at least five groups recorded similar observations in their cohorts of patients who underwent HAART. Episodes of herpes zoster, CMV, TB, but also CNS toxoplasmosis and PCP were seen in most instances.

Some authors have explained this fact by arguing that immune reconstitution in response to HAART is partial and deferred, allowing silent pathogens to become symptomatic before drug-related immune restoration can diminish their risk. However, the incidence of opportunistic events shortly after beginning therapy is even higher than expected, and many of them occur in subjects having a dramatic virologic and immunologic response. Rather than caused by a lack of protection, it has been postulated that inflammatory phenomena surrounding a rapid and profound restoration of the immune system in response to HAART in previously immunosuppressed patients could trigger the emergence of opportunistic infections in them during the first weeks of treatment. This distinct pathogenicity is in agreement with the fact that vitritis and lymphadenitis, associated with CMV and Mycobacteria respectively, are particularly common in this population, supporting that HAART can trigger inflammatory responses to opportunistic pathogens in a short period after its introduction, although thereafter the resulting immune reconstitution confers the expected protection against them.

There is no doubt that HAART provides protection or even cure from opportunistic infections but,

ironically, because of its potency, it can be deleterious in a short period after being introduced in subjects with low CD4+ counts. Clinicians should be aware that these events post-HAART can appear even in subjects without severe immunosuppression at baseline. Because inflammatory phenomena are deeply associated with its pathogenesis, the use of both corticoids and specific therapy against pathogens should be evaluated in this context.

The molecular mechanism of zidovudine resistance unraveled

The very first anti-HIV drug for which resistance had been observed to occur in cell culture and in drug-treated HIV-infected individuals was zidovudine (AZT). Although AZT resistance was unambigously shown to be due to the appearance of well-defined mutations in the reverse transcriptase of AZT-resistant HIV-1 variants, investigations have always failed to show AZT-TP (the active metabolite of AZT) resistance at the level of the enzymatic activity of the mutated reverse transcriptase. The amino acid mutations that are characteristic for AZT resistance include, but are not limited to, M4IL, D67N, K70R, T215F and K219Q. The discrepancy between the genotypic and phenotypic characteristics of mutant viruses and the phenotypic properties of the corresponding mutant enzymes is in sharp contrast with the resistance mutations in the HIV RT shown for other nucleoside RT inhibitors, and for the non-nucleoside RT inhibitors.

Recently however, the research group of Michael Parniak provided a clue that may shed more light on this controversial issue (Arion *et al*. Biochemistry, 1999, in press). These investigators found that the T215F/K219Q mutant RT had decreased template/primer dissociation from RT resulting in an increased DNA processivity. On the other hand, and more interestingly, they also found that the D67N/K70R mutant enzyme was endowed with a decreased sensitivity to AZT-TP in the presence of pyrophosphate, and an increased rate of pyrophosphorolysis (the reverse reaction of DNA synthesis) of AZT-MP chain-terminated DNA in the presence of pyrophosphate. Taking both observations together, the data strongly suggests that AZT resistance results from a selectively decreased binding of the mutant RT to AZT-TP together with an increased pyrohposphorolytic cleavage of chain-terminated DNA at physiological pyrophosphate levels, resulting in a net decrease of DNA chain termination. The observed increased processivity may compensate for the increased reverse reaction rate of the mutant RT.

It is interesting to note that this mechanism of resistance at the level of RT seems to be mechanistically different from that of other ddNTPs. As an important consequence, potent NNRTIs that inhibit the RT-catalysed pyrophosphorolysis, enable a more durable AZT-TP incorporation into the DNA when combined with AZT, and thus may prove to be a bonus to combination therapy when AZT and potent NNRTIs are combined for HIV-1 treatment.

HIV-1 group N, a new variant from Cameroon

French researchers reported in September 1998 (Simon *et al*. Nature Med 1998; 4: 1032-7) the identification of a new highly divergent HIV-1 isolate from a Cameroonian woman who died of AIDS in 1995. As noted by the same authors in 1994 regarding HIV-1 group O (Simon *et al.* AIDS 1994; 8: 1682-9), misdiagnosis of the new HIV-1 group N variant has occurred with the current commercially available serological and viral load tests. This finding is of particular importance for blood transfusion safety.

The full-length genome of HIV-1 group N was sequenced. Structural genes of the new isolate are equidistant from those of HIV-1 group M and $SIV_{cpz\text{-}gab}$, and more distant from group O, and far from HIV-2. The virus was called group N («new» or «non M-non O»). Most likely, HIV-1 group N represents a new independent cross-species transmission, as previously proposed for grup M, group O, and HIV-2. After testing sera from 700 HIV-1 positive subjects from Camerron by a specific HIV-1 group N serologic, the French authors identified 3 additional strongly reactive sera. One sample could be confirmed as group N positive by genetic analysis.

The identification of this highly divergent strain indicates that the recognition of lentiviruses in humans may not be a closed chapter, and reiterates the importance of implementing appropriate screening tests.

Role of nef in the pathogenesis of HIV in doubt

Ever since the discovery by Kestler *et al* (Cell 1991; 65: 651-62) that monkeys infected with SIV lacking Nef did not develop AIDS, suggesting that Nef is critical for the development of the disease, researchers have been investigating this intriguing HIV-1 protein. This proved to be very difficult since there is no overt *in vitro* difference between Nef-containing and Nef-delated HIV-1.

Nef-deleted HIV-1 in an Australian blood donor and several recipients of his blood seemed to have had no pathogenic effect in a time period of 14 years. The concept of a Nef-deleted vaccine is still very much debated, especially since newborn monkeys seemed to develop AIDS-like symptoms after infection with Nef-deleted SIV (Ruprecht *et al.* Science 1996; 271: 1790-2). However, one patient infected with an HIV Nef-deleted virus has shown a decline in the CD4 count (Greenough *et al.* N Eng J Med 1999; 340: 236-7). The following functions have been attributed to Nef (Harris, J Gen Virol 1996; 77: 2379-92): down-modulation of cell-sur-

face CD4 expression enhancement of virus infectivity and interference with signal transduction pathways, but the *in vivo* contribution of these functions to the pathogenesis of AIDS are not entirely known. Hanna *et al* (J Virol 1998; 72: 121-32) expressed the HIV genome under the control of the regulatory sequences of the human CD4 gene, and found that the transgenic mice developed an AIDS-like syndrome. When analysing transgenic mice expressing mutated HIV genomes, they could conclude that the major determinant for this pathogenic effect was an intact Nef gene (Hanna *et al.* Cell 1998; 95: 163-75), Larsen *et al* (J Biomed Sci 1998; 5: 260-6) and Lindemann *et al* (J Exp Med 1994; 179: 797-807) also reported that mice transgenic for the SIV Nef protein had an impaired immune system. Thus it seems that the presence of Nef alone may be sufficient to explain the main pathogenic effects of AIDS. This is a major breakthrough in AIDS research and will foster new investigations, including a search for new antiviral drugs directed towards Nef. If the function of Nef can be blocked, HIV could become a harmless virus, with less need to establish a demanding powerful combination therapy to shut down replication.