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EDITORIAL

This issue contains a report from Gareth Hardy from the Keystone Vaccine Workshop held earlier this year, and the first of a two-part article on HIV and HCV coinfection by Leighton Davies. Both these articles are longer than much of our usual coverage and follow earlier special reports on LGV in the UK, and on HPV vaccines, and we hope these are useful for readers.

This issue of HTB went to press as the IAS International was coming to a close. We have included weblinks to the conference sites and other sites that provide early coverage for readers who want to follow the programme now. Otherwise our coverage from this meeting will be included in the next issue of HTB.

Much of the conference focused on access and prevention-related issues which are also covered in this issue - including an alarming attack on a peaceful demonstration by HIV-positive people in South Africa.

Included as a supplement for this issue of HTB is a report from a meeting held earlier this year, between community treatment advocates and four Indian generic pharmaceutical companies. As well as focusing on manufacturing and pricing issues, the report highlights the different realities relating treatment for HIV-positive people in different parts of the world.

CONFERENCE REPORT

3rd IAS Conference on HIV Pathogenesis and Treatment

24-27 July 2005, Rio de Janeiro, Brasil

As we went to press the 3rd IAS Conference in Rio had just drawn to a close. We will include full reports from the meeting in our next issue but a few presentations deserve mentioning briefly here:

- The first large prospective randomised trial looking at male circumcision as an intervention to reduce female to male HIV transmission reported protection equivalent to a vaccine with 66% efficacy. [1] This association had previously only be reported from retrospective analysis. The mechanism for increased risk in uncircumcised men is thought to be due to HIV susceptible Langerhans cells in the inner foreskin, that are closer to the epithelial surface, and that have little, if any, protective covering of keratin (*See report in HTB August/September 2004*).
- Further analyses from the TOPS study evaluating single dose nevirapine "tail" coverage with 4- or 7-days of AZT/3TC (which gave significant reduction in detectable nevirapine associated resistance). This analysis included maternal viral load data showing the percentage of women with viral load <400 copies/mL in both the 4- and 7-day arms. It was suggested that this may answer the question "Why is short cover working?" The strategy (7 days cover) was included for consideration in appropriate circumstances in the new WHO draft MTCT guidelines. [2]
- Reducing resistance may also explain the findings from a study evaluating immunological response to nevirapine containing HAART in a group of women who had previously received nevirapine MTCT prophylaxis. Although the study discussed exposure to single dose nevirapine 60% of women had received AZT/3TC in addition. There was a similar response in exposed and unexposed women at six months of therapy. [3]
- *Medicins Sans Frontieres* presented encouraging results from a group of 1840 children in resource-limited settings in Africa. 97% of children were treated with WHO recommended first line therapy and 65% with cut up fixed dose combination *Triomune*. Over six-month follow up data was available for 54% of children and over one-year data for 29% of the children. The median CD4 percentage increase was +9% at six months, +11% at 12 months and +13% at 24 months. [4]

Conference coverage has been archived and is available for viewing on kaisernetwork.org with links to this site from the IAS conference website. Daily conference coverage includes webcasts and transcripts of plenary sessions, select other sessions, and the opening and closing ceremonies.

<http://www.ias-2005.org/>

and

<http://www.Kaisernetwork.org>

Abstracts from the meeting are also available to search online.

Rapid conference reports are already available on many sites including:

<http://www.hiv.medscape.com>

<http://thebody.com/>

<http://hivinsite.ucsf.edu/>

<http://www.natap.org>

<http://www.aidsmap.com>

<http://www.clinicalcareoptions.org>

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CONFERENCE REPORT

Keystone vaccine symposia 2005: vaccines, pathogenesis, snow and a couple of elk

9-15 April 2005, Banff, Alberta, Canada

Gareth Hardy PhD, for HIV i-Base

The 2005 HIV Vaccines and Pathogenesis X7/X8 Keystone Symposia, took place in Banff, Canada. Set deep in the picturesque alpine landscape of the Alberta Rocky mountains at an elevation of approximately 5,000 feet in the grand Banff Fairmont Springs hotel, the latest unveiling of cutting edge research in HIV vaccine developments and pathogenesis took place.

This latest convergence of the great and the good of HIV immunology had my anticipation of being another annual update on 'what we still can't figure out'. But instead, I came away from the meeting feeling that incremental progress is well underway, and that this may lead to substantial developments within the next few years. This wasn't because of any new clinical data on vaccines in development, or suggestions of protection in vaccinated cohorts or animal models, but because we are starting to overcome significant problematic obstacles to our understanding of HIV pathogenesis and mechanisms of immunological protection.

There is now a general consensus that a prophylactic vaccine for HIV must induce effective neutralising antibodies. All currently effective vaccines that protect against viral infections do so through elicitation of neutralising antibodies. Cell mediated responses, involving T cells, play a much greater role in the control of infection, once established and in sterilising immunity in which established infection is cleared.

In order to be effective in the long-term, neutralising antibodies have to overcome the diverse variety of mechanisms that HIV utilises to evade such immune responses to its envelope protein. These include shielding of potential epitopes by glycan molecules, conformational masking, hypervariability in immunodominant loops and occlusion of conserved regions of both gp120 and gp41. Such neutralising antibodies must be both broadly cross neutralising – in other words inhibitory to a wide diversity of different viral strains, overcoming all these obstacles – and they must be present in sufficient quantity at the mucosal surfaces where they should block infection. If an HIV vaccine candidate can meet these requirements it is more likely to be successful in protecting against HIV infection. The role of such vaccine-induced neutralising antibody responses which protect against infection, in those already infected, remain to be seen. Though it is possible that the induction of responses that protect against infection may have a therapeutic application, this is still only a possibility.

Pushing the envelope, even further

Alexandra Trkola of the University Hospital Zurich, Switzerland, presented an update on data presented at last year's conference with some additional material. In this study eight chronically-infected and six acutely-infected patients who were stopping antiretroviral treatment (ART), were treated with a cocktail of three such broadly cross neutralising monoclonal antibodies: 2G12, 2F5, and 4E10. [1]

These patients were selected for the study according to the sensitivity of their viral isolates to the three monoclonals. ART was administered for at least 3 months. Starting one day before stopping treatment, patients received 13 passive immunisations over 11 weeks, with two in the first week. Follow-up lasted for a total 24 weeks.

Two of 8 chronically infected patients controlled their viral load during the 11-week passive immunotherapy period and one controlled virus for the whole 24 week period. In contrast, far better control was seen in those patients with acute HIV infection. All 6 of these patients controlled their viral load for at least 5 weeks of the passive immunotherapy period and 2 patients

controlled viral loads beyond 12 weeks. Viral rebound in the acutely infected patients was compared to that in a control group of 12 acutely infected patients discontinuing ART.

The difference in time to viral rebound between the monoclonal antibody treated and the non treated acutely infected patients was statistically significant ($P=0.0286$). During treatment, sequential viral isolates were obtained and assessed for sensitivity to the three monoclonal antibodies. While no relevant changes were seen in sensitivity to 2F5 or 4E10, or any sequence changes in the epitopes they recognise, there was substantial resistance of rebounding virus to 2G12 in 11 out of 13 patients that originally had 2G12 sensitive virus. It was noted that the ratio of 2G12 antibody concentration in the plasma to *in-vitro* inhibitory doses were significantly higher in patients who responded, than in patients who did not ($P=0.0175$). This was despite a slightly higher dose of 2G12 compared to the other two antibodies to correct for its shorter half life.

It thus seems that variations in activity of 2G12 between individuals and therefore the dosing of 2G12 is a likely influence on the outcome of this study. Nevertheless, seven of 14 patients responded to passive immunisation with a cocktail of three broadly cross-neutralising antibodies with clearly defined delays or decreases in rebounding viraemia.

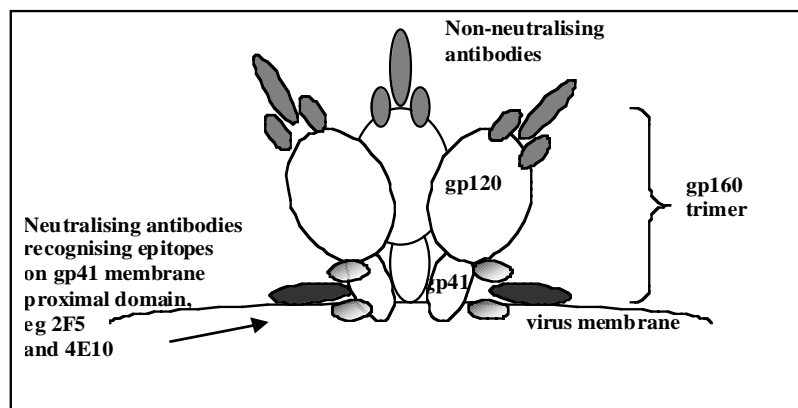
This provides the first direct evidence that these neutralising antibodies can contain viraemia in HIV-infected patients. If such antibodies could be elicited by an immunised host, then the likelihood of containing viraemia in the long-term would be far greater since production of antibodies by B cells recognising sensitive epitopes (originating from a vaccine), would have broader specificity than just the three epitopes recognised by these monoclonals.

Richard Wyatt of the Vaccines Research Centre, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD, USA, further added to our understanding of these antibodies with some rather surprising discoveries. [2]

The antibodies 2F5 and 4E10 bind to a region on gp41 called the membrane proximal region (MPR), which is highly conserved. Until recently, immunologists had considered that this space, located in the juncture between gp120 and the viral membrane, was too small for an antibody to gain access to any relevant, conserved epitopes. The number of angstroms between the underside of gp120 and the hydrophobic plasma membrane of the virus was just too few for a large antibody molecule to fit in, thus occluding the membrane proximal region.

Wyatt *et al* deployed atomic-level structural information coupled with biochemical, biophysical, antigenic and immunogenic analysis to create novel protein immunogens that are capable of generating antibodies with broadly cross neutralising activity against HIV. They used several strategies in the construction of these immunogens, including the introduction of cysteine pairs to stabilise gp120 in the CD4-bound conformation. This CD4-bound conformation reveals a greater proportion of the highly conserved membrane proximal region on gp41. Further they mimicked the membrane proximal region of gp41 to which neutralising antibodies 2F5 and 4E10 bind. Structural analysis of 2F5 with its epitope at the atomic-level, revealed a potential hydrophobic interaction between the antibody's CDR3 region and both the epitope and plasma membrane of the virion. This led Wyatt and his co-workers to investigate the binding properties of both 2F5 and 4E10 to selected Env proteins captured on solid phase proteoliposomes, with and without a reconstituted lipid plasma membrane.

These experiments demonstrated that far from the two neutralising antibodies being occluded from the membrane proximal region of gp41 by the lipid plasma membrane of the virion, they are actually dependant on the close proximity of the plasma membrane for their interaction with the region. Modified membrane proximal region 26-mers were constructed, designed on the basis of 2F5 as fusion peptides, which incorporated additional hydrophobic residues at various positions. Several of these peptides bound the 2F5 and 4E10 epitopes very well. Thus the hydrophobic domain of the CDR3 region of 2F5 allows the antibody to lie on the plasma membrane of the virus allowing 2F5 access to the occluded face of gp41, while the remainder of the antigen binding site is hydrophilic, allowing binding to the charged portion of gp41. 4E10 was found to have a greater hydrophobic region than 2F5, which Wyatt speculated would enable the antibody to actually embed itself in the viral membrane,



again giving access to the occluded membrane proximal face. These findings were somewhat remarkable, because it was previously thought that antibodies would not interact with the plasma membrane, and rather would be repelled by it.

This was one of the reasons for optimism from the meeting; at least from this presentation it is clear that the immune system is far more clever at dealing with a highly complex problem, than we sometimes give it credit for.

Discussing this with Peter Kwong, also of the Vaccine Research Centre, National Institutes of Health, Bethesda, MD, USA, who was involved with the work, he said:

"The surprising thing [in this research] relates to antibody interaction with membranes. But membranes form part of "self", and what Bart Haynes recently showed in Science was that 2F5 and 4E10 bind to a membrane component of self called cardiolipin. [3] So while the immune system is clever, the virus may be even more clever. One can either interpret Bart's results as sobering (that 2F5 and 4E10 may interact with self) or as an encouraging discovery indicating that antibodies with properties similar to 4E10 and 2F5 are naturally elicited (and that the secret to making more 2F5 and 4E10 may involve breaking mechanisms of tolerance). I favor the latter because there's no evidence that 2F5 or 4E10 cause any side effects at levels of passive immunisation which prevent HIV infection."

Adding more optimism, Jason Hammonds, of Vanderbilt University, Nashville, Tennessee, who tempted me into joining him in a mile's elevation trek to the summit of Sulphur Mountain, in which our path was crossed only by a family of elk! We discussed his latest data, while admiring the views across Banff valley from the summit. Hammonds has previously described the construction of pseudovirions which express stable gp120 trimers, altered to the CD4 bound conformation. [4, 5]

As mentioned above, the CD4 induced (CD4i) conformation of gp120 is known to reveal a greater extent of the highly conserved membrane proximal region of gp41. At this years meeting Hammonds presented his most recent data, comparing the env expressing pseudovirion against a soluble gp120 preparation, with various adjuvants including TiterMax and an Alhydrogel in Guinea pigs. [6]

High titres of anti-env antibodies were induced in these animals. In the pseudovirion immunised animals the neutralising antibody responses displayed were of a significantly greater breadth and magnitude than those from the soluble gp120 immunised animals. High titres of neutralising antibodies were found in the pseudovirion immunised animals that neutralised every strain of virus Hammonds tested. These were not only lab-adapted strains of virus which are relatively easy to neutralise but also primary isolates, suggesting that the CD4i gp120 trimer expressing pseudovirions do indeed induce production of broadly cross neutralising antibodies.

Although these animals are not a model for HIV, the fact that the pseudovirion immunogen is inducing these hard-to-generate antibodies is very encouraging. Primate, and/or human studies, are eagerly anticipated. Hammonds next stage is *"to test the pseudovirion in the SIV/macaque model"*, which he and Paul Spearman, Head of the laboratory, were hotly pursuing. This will enable verification of the antibody data in a relevant animal model of pathogenic lentiviral infection.

Dendritic cells: a Trojan horse from mucosa to lymph nodes

Turning to events that take place at the initial stage of infection, which may be blocked by novel agents, Andrew Blauvelt of Oregon Health and Science University, Portland, Oregon, discussed the role of an epithelial population of dendritic cells, langerhans cells. [7]

The role of langerhans cells in establishment of infection has remained somewhat controversial for a number of reasons. Namely, that these dendritic cells (DCs) are relatively uncommon in the mucosal epithelium, where they constitute only about 1-2% of the cells present; that their expression of HIV co-receptors changes rapidly (within hours) from CCR5+/CXCR4- to CCR5-/CXCR4+ upon maturation of the cell; and that these cells migrate rapidly (within hours) from epithelial tissue to draining lymph nodes upon exposure to HIV. Blauvelt and workers developed a skin explant model to investigate the interaction of HIV with immature langerhans cells in the epithelium and showed that langerhans cells are the initial targets of HIV following virus exposure. [8]

In order to obtain langerhans cells from mucosa, Blauvelt carried out blister inductions in healthy volunteers. Langerhans cells were derived from blister roofs in a 4 day culture experiment. Histological analysis demonstrated that the squamous epithelial cell content of this tissue was very similar to that of the vaginal mucosa and internal and external foreskin. These immature langerhans cells were co-cultivated with different strains of virus including CCR5 tropic (R5) virus (BaL) and CXCR4 tropic (X4) virus (IIIB) for a 2 hour period in which infection readily took place.

Blauvelt showed that:

- immature langerhans dendritic cells become productively infected with R5 virus by a CD4 and CCR5 dependent-process;
- R5 virus infects immature langerhans cells more efficiently than X4 virus;
- infection of immature langerhans cells by R5 virus is regulated by CCR5 polymorphisms;

- infection of immature langerhans cells of different CCR5 polymorphisms by R5 virus can be blocked by an analogue of CCR5's ligand, RANTES, called PSC-RANTES;
- in contrast to infection of CD4 T cells and macrophages, clade C HIV enters immature langerhans cells as efficiently as clade B virus;
- capture of HIV by C-type lectins, including DC-SIGN and langerin, is not a biological feature of immature langerhans cells;
- HIV-infected langerhans cells preferentially transmit virus to autologous proliferating memory CD4 T cells located in langerhans cell - T cell clusters; and
- both langerhans cell infection and langerhans cell mediated transfer of virus to co-cultured T cells can be prevented by blocking infection of immature langerhans cells.

Infection of immature langerhans cells could be prevented by incubation with the RANTES analogue, PSC-RANTES, before and during the 2 hour co-culture with virus, whereas the fusion inhibitor C34, required incubation with immature langerhans cells before, during and after the 2 hour co-culture with HIV in order to prevent infection. Blauvelt explained that immature langerhans cells from those individuals who were heterozygous for the delta32 mutation in CCR5 had a much higher degree of protection from HIV infection by PSC-RANTES, than those cells from individuals with homozygous wildtype CCR5.

One aspect of this and other similar work presented at Keystone of concern, was the lack of validation of these inhibitors in rectal mucosa models. Is the rectal mucosa much different from the vaginal mucosa? Or are there key differences in rectal and vaginal transmission? If microbicide gels containing agents such as PSC-RANTES are going to be effective, then they will have to be available as over-the-counter products. However, if we don't know that such agents are equally protective against rectal transmission, as against heterosexual vaginal transmission, then a switch in "safer sex" practices from condoms to gel could, paradoxically, have the counter-productive effect of increased rates of infection in gay men and heterosexual who have anal sex. But no-one raised this important issue; one that is commonly highlighted by community advocates around initiatives for microbicide research.

Ashley T Hasse, of the University of Minnesota, Minneapolis, Minnesota, gave the first plenary session of the X7 pathogenesis meeting, describing how a small window of opportunity exists at the very earliest stage of infection for its establishment, but which the immune response fails to close as that response is too little, too late. [9]

Hasse explained that despite the large inoculum of virus present during sexual transmission, most of that virus is cleared at the mucosa. Thus initially a very small founder population of virus gets through which has to extensively amplify itself in order to establish infection. Using the SIV model in rhesus macaques, the initial events of acute immunodeficiency virus infection were tracked following intra-vaginal infection. At 4 days from infection the tissue distribution of SIV RNA was extremely focal and extremely small, within the endocervix. After seven days there was a 70-fold expansion of SIV RNA with substantial dissemination.

At day 6 the first infected cell appeared in the mesenteric lymph nodes. Hasse explained that the virus thus follows an anatomical spread as such:

Mucosa —> Draining lymph node —> Spleen and gut

During this process, explosive SIV replication takes place in the mucosa as virus encounters resting memory (CD45RO+) CD4 T cells as well macrophages and DCs. Resting memory CD4 T cells express intermediate levels of CCR5 and act as a portal for distal virus dissemination to the lymph nodes, where activated CD4 T cells then support a massive explosion in virus replication. Thus it is described that both memory CD4 T cells and dendritic cells have roles to play in viral dissemination.

Following this, a huge loss of memory CD4 T cells takes place in the gut-associated lymphoid tissues (GALT). Here large numbers of memory CD4 T cells were found to be expressing caspase-3, an apoptosis marker, along with surface markers for apoptosis susceptibility including FAS (CD95) and FAS ligand (CD95L), suggesting that memory CD4 T cell loss in the GALT is mediated by both direct and indirect viral mechanisms. The subsequent CD8 T cell response is too late, and too small to protect against this damage, although a robust response was observed in the female reproductive organs. This was associated with a reduction to very few residual SIV infected cells in the genital tract by 28 days following infection.

A host of receptors: how would you like to enter?

Yvette van Kooyk, of the Vrije University Medical Centre, Amsterdam, Netherlands, further discussed the role of DCs in establishment and dissemination of infection. [10]

Importantly, the initial interaction of a pathogen with the DC, is crucial in determination of the type of effector T cell that differentiates in response. Recognition and internalisation of a pathogen is facilitated by specific pattern recognition receptors on the DC, namely the Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), including langerin and DC-SIGN. DC-SIGN is a CLR with affinity for high mannose carbohydrates. Though CLRs are involved in cellular processes (the natural

ligands for the CLR, DC-SIGN, are the adhesion molecules ICAM-2 and -3 which are involved in transmigration of DCs across the vascular epithelium and DC-T-cell binding, respectively), it is now known that several CLRs interact directly with pathogens. While TLRs relay information about the pathogen to the DC via signal transduction events, CLRs recognise carbohydrate structures of pathogens, including glycosylated proteins such as gp120, which they internalise for antigen processing and presentation, without induction of DC maturation.

Although these receptors have different specificities and different functions, the suggestion of cross-talk between them indicates that the nature of the immune response which results from antigen presentation by the DC, is dependant on the balanced triggering of these two families of receptors. However many pathogens have evolved strategies to evade anti-microbial and anti-viral immune responses by subverting the function of pattern recognition receptors, as excellently reviewed by van Kooyk. [11]

Van Kooyk explained the processes involved in DC differentiation. Precursor DCs enter the mucosal epithelium from the circulation, where they then reside as immature DCs. When a pathogen crosses that mucosal barrier it will encounter the immature DC through interaction with these pattern recognition receptors. Van Kooyk explained that while TLR binding initiates a sequence of events including activation of the NF-kappa-B pathway, and production of inflammatory cytokines, such as interleukin (IL)-12 and interferon (IFN)-gamma, CLR ligation results in internalisation into a lysosome where the pathogen gets degraded and processed for presentation via the exogenous class II MHC pathway. However, tissue DCs which are immature, are capable of storing unprocessed antigen unless confronted with an inflammatory stimulus. Thus while the majority of viral particles should be degraded for antigen presentation, a small amount may be protected suggesting different sorting events within the DC, which may be temporally and spatially separated *in vivo*. DCs that acquire HIV in tissues maintain a large store of unprocessed virus because the acquisition of virus and migration of the DC to the lymph node takes place under relatively non-inflammatory conditions. Captured HIV is only degraded upon maturation, during inflammation.

Van Kooyk and co-workers, speculate that HIV may silence DCs, preventing maturation and subsequent degradation. When HIV is picked up by this pathway, following gp120 capture by DC-SIGN, instead of passaging to the acidic lysosome where peptides are processed for class II MHC loading, intact infectious HIV is carried to the cell surface. This process was discussed in more detail in last years Keystone report. [5] Van Kooyk demonstrated that infectious virus remains stabilised in this way for as long as 4 days, in which virus is co-localised at the infectious synapse. Despite this, capture of HIV by DC-SIGN can lead to virus processing and presentation. Using an anti-DC-SIGN antibody, van Kooyk and colleagues showed that HIV-infected DC stimulation of a gp120-specific CD4 T cell line was inhibited by as much as 50%, thus DC-SIGN is considered to be involved with antigen presentation after pathogen internalisation. Furthermore recent published work by van Kooyk *et al*/shows that X4 virus may be captured and internalised by DCs in 2 hour cultures regardless of the presence of DC-SIGN. [12]

Thus while DC-SIGN contributes to HIV binding, it does not contribute to HIV capture and internalisation. In contrast the formation of the infectious synapse between X4 virus infected DCs and uninfected resting HeLa P4-2 CD4 T cells was significantly impaired when DC-SIGN expression was inhibited with a specific short interfering RNA (siRNA). Thus in the absence of CCR5 utilisation, HIV is equally capable of infecting in trans, DC-SIGN-ve and DC-SIGN+ve DCs, although DC-SIGN is important in initial virus binding. Therefore other internalisation pathways in addition to DC-SIGN participate in viral capture by DCs.

Futhermore, while DC-SIGN is not required for formation of DC-T cell clusters, the formation of the infectious synapse between DC and T cell is somewhat dependent on DC-SIGN, and DC-SIGN is unexpectedly very much present in that synapse. This demonstrates that DC-SIGN has an important role to play down stream of viral capture.

Work using other viruses, such as measles, which utilise the same DC-SIGN pathway, shows that measles virus uptake by DC-SIGN induced an IL-10 phenotype. Such a phenotype induces clonal tolerance in specific T cells, and the induction of T-regulatory cells. Van Kooyk explained that both probiotics, such as certain lactobacilli, and self glycoprotein antigens target CLRs inducing peripheral tolerance. Thus pathogens which target CLRs, including DC-SIGN, Mannose receptor (MR) and Dectin-1, may be subverting the function of those CLRs, whose physiological function is to recognise self antigens. The mannosylated lipoarabinomannan (ManLAM) component of the cell wall of *M. Tuberculosis*, and a variety of other mycobacteria, also utilise the DC-SIGN pathway to a similar end. In such a setting the balance of expression of TLRs and ManLAMs on the DC dictates whether a type 1 (TH1) or type 2 (TH2) response is elicited. Van Kooyk concluded that the utilisation of specific CLRs and TLRs on the DC can switch a TH1 or Th2 response, or tolerance, appropriately or inappropriately. Thus in terms of vaccine design, it is now clear that not only must vectors be designed to target DCs, but they must be designed to target DCs in a particular way, utilising specific CLR/TLR combinations, which finally we are starting to get a real grip on.

In alternate primate hosts, the consequences of DC entrance dictate life and death!

Mark Feinberg, of Emory University School of Medicine and Emory Vaccine Center, Atlanta, Georgia, USA, followed on this story with some very nice work in primates, highlighting important distinctions between diverse clinical outcomes in different species. [13]

Sooty mangabeys are the natural hosts for SIV infection. Despite high levels of plasma viraemia in these animals, there is no CD4 T cell depletion, no elevation of CD4 or CD8 apoptosis, no increased CD8 T cell proliferation and no disease progression. Interestingly depletion of CD8 T cells in these animals had no effect on viral load, suggesting that the limited SIV-specific CD8 T cell responses in these animals had no effect on viral activity. Interestingly these animals are fully able to respond to and control other viral infections. Therefore it appears that in sooty mangabeys a relative state of clonal non-responsiveness exists, and that despite high viral turn-over - sometimes greater than in HIV infection in humans and pathogenic SIV infection in other primates e.g. rhesus macaques - the lack of immune activation in these animals is the principal condition which correlates with disease protection.

To better understand the cellular and molecular basis of whether or not chronic immune activation and immunopathology follow immunodeficiency virus infection, Feinberg and colleagues studied divergences in the innate and adaptive immune responses to SIV in sooty mangabeys and rhesus macaques respectively. Differences in DC activation and migration in response to SIV were apparent within the first days of infection, which were subsequently followed by substantive differences in the magnitude and type of adaptive immune response.

These differences in *in-vivo* responses were mirrored following *ex-vivo* exposure of sooty mangabey, rhesus macaque and human plasmacytoid dendritic cells (pDCs) to specific TLR ligands and to inactivated virus. pDCs of sooty mangabeys failed to mature or express CCR7, which would home them for the lymph nodes, upon exposure, in contrast to the pDCs of humans and rhesus macaques. In addition there was also a significant diminution in production of IFN-alpha. Feinberg explained that this was apparently the result of divergent propagation of activation signals along post receptor pathways. While both sooty mangabeys and rhesus macaques were able to produce IFN-alpha-2 in response to influenza virus, only rhesus macaques produced IFN-alpha-2 in response to inactivated SIV. At the organism level, gene expression profiling studies further indicated that a major feature which distinguishes pathogenic from non-pathogenic immunodeficiency virus infection is the extent to which a pattern of type-1 interferon production and response profiles manifest. Interestingly Feinberg pointed out that Type-1 interferon genes were amongst the most strongly up-regulated in T cells of HIV infected humans, in stark contrast to SIV infected sooty mangabeys.

Feinberg concluded that a genetically programmed generative stage failure of sooty mangabey innate immunity to respond to SIV infection, manifest as lack of pDC maturation and migration to the draining lymph nodes, represents the first divergence in host immunity between species, which determines differing infection outcomes between these species.

Sara Kluckling, also of Emory University, Atlanta, Georgia, USA, further added to this line by showing the results of experiments in which pDCs of sooty mangabeys and rhesus macaques were stimulated via TLR-9, with CpG. [14]

Again, pDCs of rhesus macaques responded by expressing IFN-alpha, where as sooty mangabeys did not. However there were no differences in expression of other cytokines tested, IL-6, IL-12 or TNF-alpha. Additionally using this stimulus there were also no differences in co-stimulatory molecule expression, CD80 and CD86, or in lymph node homing receptor, CCR7 between species' pDCs.

T cell responses in chronic HIV infection: a different set of problems, with a different set of solutions

Following my concern on the subject after last year's Keystone meeting, it was good to see the IL-2/IFN-gamma story getting more mileage this year, as a replacement for IFN-gamma single parameter measurements. Building on the work of Marc Boaz in London, who originally described this dual phenotype in long-term non-progressors, Souheil-Antoine Younes in Montreal and Alexandre Harari in Lausanne have confirmed that this phenotype of antigen-specific T cells is a useful correlate of immunity, at least in infected individuals. Complex multi-colour technology is gradually unravelling a not very clear picture of T cell differentiation, in mice and men differentially, using markers such as CCR7, CD45RA, CD62L, CD27 and CD28.

In addition CD127, the IL-7 receptor alpha chain, has also entered the fray as a likely contender for the distinction of small numbers of short-lived effector T cells which have a tendency to survive into the long-lived central memory T cell pool, although the directional differentiation between these subsets is debated. Such T cells are now being considered critical components of protective T cell responses, which are thus likely to become correlates of immunity in cohorts of "protected" patients. However, leaving aside the high-tech revolution in multi-colour flow cytometry, an alternate handle on the same, or similar, effector memory T cell responses, liable to generate long-lived central memory, is the dual IFN-gamma/IL-2 expression phenotype. In larger scale vaccine trials an assay, be-it ELISpot or flow-based, for this dual cytokine phenotype may represent a high through put alternative to complex multicolour flow technology, which is not available in many parts of the world.

Steven Deeks, of the University of California, San Francisco, CA, USA, described T cell responses in a cohort of patients who maintain low-level viraemia in the presence of high-level drug resistance, ("partial controllers on antiretroviral therapy", PCAT). [15]

In these patients, Deeks and co-workers observed that:

- HIV is often constrained in its ability to develop high-level drug resistance while maintaining replicative capacity;
- immune activation is reduced in patients with drug-resistant virus, in comparison with patients with similar viral loads composed of wild type virus; *and*
- HIV-specific T cell responses are often very high during incomplete viral suppression.

Deeks explained that the immunologic characteristics of this group of patients were very similar to those of long-term non-progressors. Low levels of activation and spontaneous proliferation were once such parallel. Surface expression of the activation markers CD38 and HLA-DR on CD4 T cells of patients with multiple drug resistant virus were significantly lower than those of patients with wild type virus and similar viral loads. Another such shared characteristic between long-term non-progressors and PCATs were well-preserved HIV-specific IL-2 and IFN-gamma-high producing CD4 T cells. Deeks showed that the percentage of CD4 T cells which responded to HIV gag with a dual IFN-gamma/IL-2 phenotype in long-term non-progressors (n=17) was significantly greater at about 0.4-0.5% of lymphocytes, than in patients receiving HAART whose virus was fully suppressed (n=40) at about 0.05-0.1% of lymphocytes ($P=0.01$). When looking at patients with multiple drug resistant virus, who partially control virus, the percentages of IFN-gamma/IL-2 co-expressing gag-specific CD4 T cells were equivocal with long-term non-progressors. Deeks concluded that control of viraemia in PCATs is associated with an IFN-gamma/IL-2 CD4 T cell response as seen in long-term non-progressors, and that both groups of patients are able to maintain this population without exhausting the CD4 response.

Turning to CD8 T cell responses Michael R Betts of the Vaccine Research Centre, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, MD, USA, presented his work on polyfunctional T cell phenotypes in long-term non-progressors and progressors at one of the afternoon workshop sessions. [16]

This work was further expanded on by Richard A Koup, Head of that lab, in one of the plenary sessions. An abundance of evidence now clearly implicates CD8 T cell responses in protection from disease progression and control of viral replication in HIV-infected individuals. Although these responses are thought to play a role in long-term non-progression, Betts points out that the magnitude of CD8 T cell responses between progressors and non-progressors is not notably different and few comparative differences in CD8 T cell responses between the two groups have been identified. Betts explained that using 11 parameter flow cytometry his group analysed the CD8 T cell responses of 9 long-term non-progressors and 79 progressors.

They identified a five-function panel in CD8 T cells consisting of the inflammatory cytokines IFN-gamma, IL-2, TNF-alpha, the chemokine MIP1-beta and the degranulation marker previously described by Betts, CD107a. Using Flow Jo software, 31 potential populations were possible with these 5 parameters. They found that long-term non-progressors maintained a polyfunctional CD8 T cell response with 4 or more of these 5 markers in response to HIV proteins: gag; pol; env; and tat/rev/vif/vpr/vpu. This response tended to include IFN-gamma, TNF-alpha, MIP-1-beta and CD107a with or without IL-2. In response to HIV antigens, the 5 function phenotype consisted of approximately 10% of the CD8 T cell response. This response was markedly deficient in progressors and there was no improvement after the first few months of HAART.

Betts plan to assess these responses in patients who had been on longer term stable HAART as an important aspect of their follow up. Interestingly Betts and his team also found that this response was a normal component of the CD8 T cell responses against CMV, EBV and flu in both progressors, long-term non-progressors and uninfected individuals.

Rick Koup expanded on this work describing that the 5 functional, IFN-gamma/ IL-2/TNF-alpha/MIP-1-beta/CD107a, population had a trend towards, but was not exclusively, a central memory phenotype (CD45RO+/CD27+/CD57-). While IL-2 expression tended to be the main difference between 5 and 4 function CD8 T cell responses, as the number of functions dropped to 3 functions the cell surface phenotype tended to become more of an effector type (CD45RO+/CD27-/CD57+/-). Koup described some investigations of CD4 T cell responses with these parameters, and explained that the proportion of IFN-gamma/IL-2 expressing CD4 T cells was significantly higher in long-term non-progressors than progressors. In cohorts of DNA and adenoviral HIV vaccinated patients, a major component of the polyfunctional CD4 T cell response tended to comprise IFN-gamma and IL-2, without TNF-alpha. This was in contrast to other cohorts of CMV infected or vaccinia virus immunised subjects who elicited a mainly IFN-gamma/IL-2+/TNF-alpha+ response. Thus, in conclusion, although similar quantities of HIV-specific CD8 T cells may be apparent in both long-term non-progressors and progressors, long-term non-progressors have a qualitatively superior CD8 T cell response to HIV. Consideration of multiple parameters of functionality are important in the determination of protective responses, both with regard to CD4 and CD8 T cell responses. While this data confirms that IL-2, together with IFN-gamma, is an important co-feature of protective CD4 T cell responses, it is evident that MIP-1-beta is likely to be at least as equally an important feature of polyfunctional CD8 T cell responses.

The implication of this, is that single parameter measurements of T cell function in vaccine and immunotherapy trials are becoming outmoded, partly by technological developments, but more so by a realisation that what constitutes a protective T cell response is likely to involve multiple simultaneous functional parameters, that must be co-incident. For the purposes of simply defining the numbers of antigen-reactive T cells in antigenicity studies, single parameter IFN-gamma assessment is a good choice as an endpoint. But for vaccine trials and studies which seek to identify correlates of protective immunity, and to determine the nature of responses we must induce in infected patients and alternately in unexposed populations in whom

we wish to confer protection from infection, the board is thrown open to a diversity of players. Such studies will need to include measurements of polyfunctionality in CD4 and CD8 T cell responses, in order to determine which are the protective phenotypes.

We are starting to get a real handle not only on what kind of responses to look for, but possibly also on how to go about manipulating the generation of those responses. On many fronts, I sensed hope. I flew home, still somewhat jet-lagged, and a little weary from the altitude and dry air, content in the knowledge that exciting progress is being made, and that somewhere, amongst the fir trees, a young family of elk are seeing the coming of summer through the winter snows.

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SPECIAL REPORT

HIV/HCV coinfection – part 1

Leighton Davies MD, MSc for HIV i-Base

This is the first of a two-part article. Part two will be included in a future issue of HTB and will focus on clinical management including treatment and controversies in HIV/HCV co-infected patients.

Epidemiology

Co-infection with HIV and HCV is common in Europe and the USA affecting approximately 30% of HIV-infected individuals and 10% of HCV-infected patients, respectively. [1] Approximately 170 million people worldwide have chronic HCV infection, including 17 million people with HIV/HCV coinfection. In Europe the incidence of co-infection in HIV-positive patients varies by countries from less than 5% (Netherlands) to around 50% (Spain, Italy). Because HCV is a blood borne virus, incidence is highest where HIV has primarily been driven as an IVDU epidemic. This figure is likely to be higher still in some Eastern European countries.

The high prevalence of co-infection is attributed to common risk factors for transmission - blood and blood products. HCV is much more infectious than HIV by percutaneous blood exposure, being transmitted by 15 to 30 of every 1000 needlestick injuries, compared with 3 per 1000 for HIV. [2] HCV can remain infectious in dried blood for several hours and replication competent virus was reported in dried blood after several weeks in one study (although those conditions were unusual). HIV becomes uninfected in less than a minute outside the body. Replication rates of HCV are much higher than for HIV, resulting in higher viraemia, which itself is a factor for transmission.

In the UK, the prevalence of HCV co-infection is high amongst intravenous drug users (IVDUs) (91%), or people who used transfusion of blood or blood products (71%) prior to 1985, when heat treatment of blood products was introduced. Even though HCV was not discovered until 1988, and a test for HCV was not marketed until 1991, heat treatment was effective at preventing infection with HCV.

Several large studies have shown that the prevalence of sexual transmission is negligible between monogamous heterosexual partners. [3] Sexual transmission is higher (estimates 4-8%) among HIV-positive gay men and men who have sex with men (MSM), who do not otherwise have traditional risk factors for acquiring HCV. London HIV clinics have collectively reported data on several hundred HIV-positive MSM who have become infected with HCV in the past 2-3 years and this is now regarded as an epidemic of HCV in HIV positive men in South-East England. [4, 5]

Risk factors for sexually acquired HCV, in addition to HIV, include sexual practices that have higher risk of trauma, including brachio-proctal stimulation (fisting), receptive and insertive anal intercourse without condoms, group sex and recreational (non-IV) drug use including cocaine and ketamine. It is speculated that these drugs when administered intranasally may facilitate the transmission of HCV through traumatic nasal mucosal damage and traces of blood on shared drug paraphernalia. Given the infectiousness of HCV, this is plausible, but verified cases of transmission have not been documented through this route. High HCV acquisition rates in HIV positive MSM have been linked to reports of lymphogranuloma venereum (LGV) infection, for example in the recent cluster reported in Rotterdam. [6] There is therefore accumulating evidence that HCV is a sexually transmitted infection amongst HIV-positive MSM. The presence of other sexually transmitted infections seems to enhance the infectivity of HCV. [7, 8] HCV viraemia is higher in co-infected patients, and correlates with that in semen, which may also facilitate sexual transmission. [9]

Vertical transmission accounts for 2-5% of infants born to HCV-infected mothers becoming infected with HCV [10], which increases to 17-20% if the mother is co-infected with HIV. Unlike HIV, however, there is no association between breast-feeding and acquisition of HCV, unless the mother's nipples are cracked or bleeding. [11]

Natural history and diagnosis

Around 15-40% of people monoinfected with HCV spontaneously clear the virus, although this occurs less frequently in people who are coinfected with HIV, and this may be related to CD4 count at infection. If it is not spontaneously cleared, HCV infection enters a long chronic phase during which 20-30% of immunocompetent patients will progress to cirrhosis after 15-30 years. HIV coinfection increases both the incidence and rate of HCV disease progression.

HCV infection can be diagnosed with a second or third generation screening enzyme immunoassay (EIA) in HIV-co-infected individuals, however this assay frequently produces negative results with low CD4 counts or after spontaneous clearance. The gold standard, in terms of sensitivity and specificity, in diagnosing HCV infection – particularly in immunosuppressed populations – is HCV RNA PCR. It is also necessary to use PCR distinguish a prior resolved infection from chronic infection. Because HCV RNA can occasionally be detected intermittently, diagnosis and treatment decisions should not be based on the result of a single PCR result. Quantitative PCR are generally more sensitive than qualitative PCR, and currently have a lower level of detection of 200 copies/mL.

Quantitative RNA testing, unlike HIV-1 RNA testing, does not offer prognostic information on HCV disease. Its use lies in stratifying the response to anti-HCV therapy, with lower viral loads (<800,000 MIU) generally being associated with higher response rates. Normal range for HCV viraemia is several logs higher than for HIV disease.

Current recommendations call for testing of all HIV positive individuals at time of diagnosis and periodically (~6 monthly) for those considered to be at higher risk (high risk sexual practices, intravenous drug use) by HCV antibody, with subsequent confirmation of viraemia by HCV RNA PCR. In patients with unexplained liver disease with a negative HCV antibody test, PCR should be performed. [12] However as the HCV antibody EIA often generates false negative results, community organisations have called for RNA PCR testing to be the standard of care and is increasingly being adopted as such in most UK centres.

There are 6 main genotypes of HCV, with over 100 subtypes. Type 1 is the commonest in the USA, accounting for over two thirds of infections. Types 2 & 3 are more common in Europe. Type 4 has a predilection for infections in Egypt.

HCV genotype is the most significant determinant in response to achieving sustained virological response (SVR) after treatment regardless of HIV status, with genotypes 2 and 3 being more sensitive than 1 and 4.

Genotyping of all individuals should be performed at the time of first diagnosis, and in cases where exposure to HCV is suspected, the genotype may change over time through reinfection, and should be retested periodically. [12]

Clinical course

Acute HCV infection is usually asymptomatic, although a typical presentation of hepatitis with jaundice (in about 20% patients), anorexia, malaise and elevation of hepatic transaminase enzymes may be seen. High levels of on-going viraemia are commonly observed, with more than 10 trillion viral particles per day being produced (compared with 10 billion per day with HIV). [13]

If HCV is spontaneously cleared, and HCV viral load becomes undetectable, ALT/AST levels normalise and anti-HCV antibodies may persist for many years. It seems that adaptive immune responses are delayed, raising the hypothesis that the virus 'outpaces' the immune system. Accordingly, clinical symptoms such as jaundice, attributable to T-cell mediated liver injury are rarely observed in acute HCV infection.

Chronic HCV infection is often asymptomatic, with normal transaminases despite persistent viraemia. Alanine aminotransferase (ALT) has been considered the most sensitive liver enzyme for HCV infection, although the severity of infection does not correlate well with ALT elevation, which may vary considerably from month to month.

Additionally, people with normal ALT can have serious liver damage, and ARV drugs can elevate ALT. Neither ALT or HCV RNA indicate or predict severity of liver disease very clearly and this is both counter-intuitive and confusing given the reliance on surrogate markers in the management of HIV. Elevated serum bilirubin levels and prothrombin times, with depressed albumin and platelet levels, signify more advanced hepatic disease.

Although recent research into using non-invasive ultrasound, serum biomarker panels and fibroscan are looking to reduce reliance on invasive procedures, these have not been validated in coinfecting patients. Liver biopsy remains the gold standard for assessing the grade and stage of liver disease.

UK guidelines recommend that use of biopsy should balance the risks and benefit for each individual. Many centres feel that the risk of a liver biopsy outweighs the benefit in people with bleeding disorder, although this also depends on the experience of the doctor performing the biopsy. There remains debate on the value of biopsies in HIV infection, even in those without haemophilia or similar disorders.

Mild liver damage is classified as a modified Ishak score of 3 or less and a fibrosis score of 2 or less. Moderate liver damage has an inflammatory score of 4 or more and/or a fibrosis score of 3 to 5. The clinical severity of advanced liver disease can also be graded by scores such as the Child-Pugh system. With the improved results of treatment with pegylated interferon for genotypes 2 and 3 many physicians may consider treatment without liver biopsy for those infected with these genotypes. [12]

Of the 85% developing chronic infection approximately 70% will have an indolent course, with low ALT elevation and progression to cirrhosis over several decades, if ever. Fatigue and depression are the two most common symptoms of chronic HCV.

Approximately 20% of people with chronic HCV will develop cirrhosis over an interval of 15 to 20 years, some may develop cirrhosis much later and others will not develop any significant liver damage. [14] HIV also accelerates HCV progression.

A UK study also reported that 15% HIV-positive MSM who are sexually infected with HCV may have a more severe course with rapid disease progression, often as early as 6 years post-infection. [15] Of those developing cirrhosis about 1-4% each year will go on to develop hepatocellular carcinoma (HCC). [16] HCV accounts for approximately 40-50% of liver related deaths in the USA and Europe and is the leading cause of liver transplants in these countries. Factors associated with fibrosis progression are duration of infection, male gender, age >40 years, older age at infection, alcohol consumption >50 grams/day and HIV co-infection, especially among persons with <200 CD4 cells/mm³— this population is at greatest risk for serious liver damage.

Hepatocyte damage is not thought to be mediated by direct viral cytopathic effects but through induction of apoptosis in HCV infected cells. However, the molecular mechanisms that cause liver cell damage during HCV infection have not yet been fully and clearly defined.

Early studies in the pre-HAART era failed to show that HCV accelerates HIV progression as assessed by immunological [17] or clinical parameters [18], although HCV may act as a co-factor for HIV disease progression in several ways: non-specific immune stimulation driven by chronic HCV infection may enhance HIV replication; the infection of immune cells by HCV could favour the depletion of the CD4 cell pool and partly blunt the immune recovery that follows successful anti-retroviral therapy; and finally, HCV could compromise the benefit of HAART as a result of a higher incidence of hepatic toxicity and therapy discontinuation. Indeed the Swiss HIV Cohort study demonstrated that HCV infection was independently associated with an increased risk of progression to AIDS or death, despite a similar use of HAART as those mono-infected HIV positive individuals. It also suggested that co-infected patients were less likely to achieve a CD4 count rise of at least 50 cells mm³ within 1 year than their mono-infected counterparts. The virological response to HAART was, however, not deemed to be affected by HCV co-infection. [19] There is compelling evidence that HIV, however, accelerates the course of HCV infection and this will be discussed subsequently.

Immunopathogenesis

Research into HCV pathogenesis has been hampered by the lack of small animal models of infection – the nearest animal model being the chimpanzee, which is an endangered species and therefore expensive. Some success has been obtained by producing HCV replicons in hepatoma cell lines [20], allowing for the expression of the complete HCV open reading frame, encoding for both structural and non-structural proteins. Another approach has been to transfect SCID mice with human

hepatocytes [21], which supports productive HCV infection.

Finally, a different approach has been recently developed: laser capture microdissection (LCM) technique allows the isolation and analysis of single infected cells chosen by histological and immunohistochemical criteria from liver sections. [22]

The mechanisms responsible for tissue injury in acute and chronic infection are not well understood. HCV is generally not considered to be a cytopathic virus because of the absence of classic cytopathic features in liver biopsy samples, although HCV appears to have important interactions with host cell proteins that might adversely affect hepatocyte survival or regeneration. The classic understanding of the pathogenesis of liver disease is that it is due to the cellular immune response against the virus, including that of CD8+ cytotoxic lymphocytes (CTLs), which activates hepatic stellate cells, leading to inflammation and fibrosis.

There is little evidence to suggest a direct virological interaction between HIV and HCV in the liver. HIV is lymphotropic, but does not infect hepatocytes directly. HCV, on the other hand appears to be lymphotropic as well as hepatotropic as viral sequences have been amplified from peripheral blood mononuclear cells (PBMCs) of infected individuals. [23] Overall, infection of non-hepatocytic cells may constitute a 'reservoir' that would favour selection of HCV variants and viral persistence. In fact the almost universal recurrence of HCV infection after orthotopic liver transplantation corroborates the existence of extra-hepatic sites where HCV can persist and replicate.

HCV is a small RNA virus of the genus Hepacivirus and is a member of the Flaviviridae family, previously termed "non A-non B hepatitis virus" before it was first identified in 1989. Very little is known about the mechanism of entry of the HCV into its target cell – the hepatocyte. Some limited evidence suggests that this may be partially mediated by binding to a receptor complex that probably includes the ubiquitous tetraspanin CD81 and as-yet-unknown hepatocyte-specific factors. Further research into identifying the precise mechanism of cellular entry is warranted as potential therapeutic agents akin to the fusion inhibitors and CCR5/CXCR4 antagonists of HIV could be developed.

Following infection of a hepatocyte and internalisation, the 9.6-kb positive single-stranded RNA genome comprising the coding sequences of the structural proteins (C, E1, E2, and p7) and the non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, NS5B), is uncoated and undergoes two fates:

- i) It is translated by host cellular ribosomes into a long polyprotein, which is subsequently cleaved by both host (signal peptidase) and virus derived (NS2/NS3 metalloproteinase, NS3/NS4A serine) proteases to form mature viral proteins. These proteins comprise the structural components, which constitute the viral particle, and the non-structural proteins that are generally involved in protein processing and genome replication.
- ii) The virus-encoded RNA-dependent RNA polymerase (NS5B) replicates the HCV RNA genome is replicated by into new genomic strands, packaged with structural proteins into mature viral particles. These particles are then released by cell lysis or exocytosis.

It is becoming apparent that HCV may circumvent the host's innate anti-viral response at several levels:

- Inhibiting genes that stimulate interferon production;
- Inhibiting interferon signalling; *and*
- Blocking interferon-inducible protein kinases.

Three possible mechanisms have been proposed to account for this resistance to IFN-alpha and IFN-beta (the so-called Type 1 interferons):

First, the HCV serine protease NS3-NS4A blocks interferon regulatory factor-3 (IRF3)-mediated induction of type 1 IFN. [24]

Second, specific sequences within E2 and the NS5A proteins seem to inhibit the activity of RNA-stimulated protein kinase R (PKR), which is responsible ultimately for inhibiting viral RNA and protein synthesis. [25] Intriguingly E2 sequences of HCV genotype 1 appear to inhibit PKR more efficiently than E2 sequences of HCV genotypes 2 and 3.

Finally, specific HCV proteins might interfere with the function of innate effector cells, such as natural killer (NK) cells. Recent in vitro studies have shown that high concentrations of recombinant E2 crosslink the tetraspanin CD81 at the surface of NK cells, inhibiting their cytotoxicity and cytokine production. [26]

Patients who spontaneously recover from HCV typically mount vigorous multi-epitope-specific CD4+ and CD8+ T-cell responses that are readily detectable in blood samples. By contrast, patients with chronic HCV tend to have late, transient or narrowly focussed T-cell responses. [27]

Individuals who have a polyclonal HCV-specific CD4+ cell response are more likely to clear HCV, as opposed to individuals who do not (e.g. HIV infected persons); and are more likely to become persistently infected. As with the CD4+ cell response, polyclonal and multi-specific CD8+ CTLs are also associated with spontaneous clearance – whereas a more narrowly focussed response during acute infection tends to lead to chronic infection.

Recent studies have suggested that there is functional “stunning” of the immune response in acute HCV infection, with impaired production of IFN-gamma by virus-specific CD8+ cells, and that this persists in patients with chronic HCV infection.

The normal, uninfected liver maintains a largely tolerogenic environment and contains a large number of intrahepatic T-cells. Why this normally tolerogenic environment should change to an inflammatory one is currently unclear. [28]

It is clearly established, however, that HCV mainly produces persistent infection accompanied by a viral escape mechanism through viral sequence mutations. HCV RNA exhibits significant genetic variability, with a mutation rate of 1 in 1000 bases per year, in all its domains producing populations of ‘quasi-species’. These quasi-species may favour the selection of RNA molecules that are ‘resistant’ to anti-viral factors. Its quasi-species nature, comparatively high replication rate and lack of proofreading capacity of its RNA polymerase all contribute to a rapid diversification of the viral population.

At the T-cell level, such viral escape seems to affect epitope processing, MHC binding, and T-cell receptor (TCR) stimulation. Very recent findings have challenged conventional thinking that the large numbers of sequence mutations were simply random in the virus’s ever-changing genome.

This new research suggests that Darwinian genetic selection is at play – the virus’s genome changes in ways that render it reproductively more fit in the face of each immune system it encounters. Stuart Ray and colleagues from the Johns Hopkins University, found that when the immune response weakens, the virus naturally mutates towards a set of 3,000 common amino acids – the virus’s “preferred” state. During the acute phase, under intense immune pressure, the virus is forced to drift away from an ancestral set of sequences (the consensus sequence), using mutations to evade the immune system. Once the virus successfully evaded a particular immune cell, its amino acids reverted back to the consensus set. Thus it is proposed that this genetic drift is the mechanism for how the virus escapes the acute immune response and establishes a chronic state of infection. [29]

The effect of HIV on HCV cellular immune responses

Numerous studies have demonstrated that patients with HIV have a higher rate of progression of fibrosis, especially those with CD4 cell counts less than 200 cells/mm³. Before the advent of HAART, patients co-infected with HIV and HCV had an approximate 3.6 fold increase in risk of developing cirrhosis. In the HAART era, End Stage Liver Disease (ESLD) resulting from HCV coinfection has emerged as a leading cause of death among HIV-positive people. Reconstitution of immunity may lead to a decrease in the rate of progression to fibrosis and risk of clinical events due to liver disease. However, this is still controversial as some people die from HCV complications at high CD4 cell counts on HAART.

Apoptosis in HCV infection

Both with liver damage and oncogenesis, a disturbance of apoptosis has been implicated. HCV-triggered liver injury is mediated mainly by host immune mechanisms. Caspases are enzymes that are involved in the final steps of apoptotic signalling pathways, and their activation is postulated to be triggered by death ligands. Other cytokines, granzyme B or HCV proteins, appear to be closely correlated with the immune response. There is growing evidence that death receptor mediated apoptosis plays a critical role in HCV-associated liver injury. The Fas/Fas ligand (or CD95/CD95L) death receptors probably have the most pathogenic role. Similarly Tumour Necrosis Factor (TNF) has been shown to activate effector caspases efficiently in HCV infection. Besides death receptors, the granzyme B/perforin pathway almost certainly plays a role in HCV-mediated apoptosis.

Thus activated CTLs recognise viral antigens by the TCR in the context of MHC antigens. TCR activation induces the expression of death ligands, such as CD95L or TNF-related apoptosis-inducing ligand (TRAIL), which bind to their cognate receptors on hepatocytes and trigger caspase-8 activation. Simultaneously CTLs release cytotoxic granules containing granzymes and perforins. Internalised granzyme B can directly activate caspase-8 and other caspases. Both mechanisms converge in a mitochondria-dependant pathway at the level of executioner caspases, which cleave various proteins and lead to cell death.

Interferons produced upon viral infection may modulate apoptosis by a number of mechanisms, such as activation of the JAK-STAT pathway, PKR, the 2', 5' oligoadenylate system (in particular RNase L); or upregulation of TRAIL and its receptors. Moreover, HCV proteins, in particular the core protein, may either positively or negatively regulate cell death. The HCV core protein has been reported to sensitise cells to death-receptor-mediated apoptosis, whereas it may exert inhibitory effects through the activation of NF-kappa-B and subsequent induction of anti-apoptotic gene products including Bcl-X_L, Bcl-2 and inhibitor of apoptosis proteins (IAPs). The pro-apoptotic pathway may be involved in liver damage, whereas inhibition of apoptosis may contribute to viral persistence and development of hepatocellular carcinoma. Recently it has been demonstrated that the E2 protein, in conjunction with HIV gp120, induces apoptosis in hepatocytes via a Fas-FasL-dependant pathway, which ultimately leads to the release of cytochrome c from mitochondria, as well as activation of downstream apoptotic signalling cascades. [30]

Hepatic fibrogenesis

The alterations in cytokine production that accompany HIV infection may aggravate the already blunted endogenous interferon response seen in chronic HCV infection. Liver fibrosis (termed cirrhosis when it affects the entire liver parenchyma) is a dynamic process involving complex cellular and molecular mechanisms initiated by chronic inflammation due to liver-tissue damage, led by the activation of quiescent hepatic stellate cells (HSCs), and resulting in the re-modelling and deposition of the extra-cellular matrix (ECM).

Fibrosis results from excessive accumulation of ECM. The collagens are the most important molecular targets: since they represent the major matrix proteins; they form important mechanical cytoskeletal scaffolds; and their proteolysis by specific proteases appears to be rate limiting for ECM removal. The fibril forming interstitial collagens type I and III, and the sheet-forming basement membrane collagen type IV are the most abundant ECM components in the liver. In cirrhosis their content increases up to 10-fold. Adverse stimuli, including viruses, toxins, hypoxia, and bile stasis can trigger fibrogenesis either by cytokine release or simply by mechanical stress.

In the acute phase of liver disease, fibrogenesis is balanced by fibrolysis – the removal of excess ECM by proteolytic enzymes, the most important of which are the matrix metalloproteinases (MMPs). MMP-1, -2, -3, -8, -9, -12, -13 and -14 are expressed in human liver cells. With repeated injury of sufficient severity, fibrogenesis prevails over fibrolysis, resulting in excess ECM deposition, i.e. fibrosis. Fibrogenesis is characterised by an upregulation of ECM synthesis, a downregulation of MMP secretion and activity and by an increase of the physiological inhibitors of the MMPs, the tissue inhibitors of MMPs (TIMPs). Collagens, MMPs and TIMPs are mainly produced by myofibroblastic cells, which are derived from HSCs or from activated (portal and perivascular) fibroblasts. Activated liver macrophages (Kupffer cells) or proliferating bile ductular epithelia or endothelia, other mononuclear cells and myofibroblasts themselves are sources of fibrogenic cytokines and growth factors that can stimulate HSC and perivascular fibroblasts to become MFs. [31]

The severity and progression of fibrosis in chronic liver disease is exacerbated by additional factors that include alcohol use, and, of particular relevance to HCV/HIV co-infection, Non-Alcoholic Steato-Hepatitis (NASH). This is a disorder related to the metabolic syndrome (hypertriglyceridaemia, insulin resistance, obesity) which has recently been closely associated with exposure to NRTI therapy and with stavudine use in particular. [32]

There is an increased risk of diabetes among people with HCV, and this has also been reported with use of use of protease inhibitors among coinfecting people.

Hepatocellular carcinoma

The precise mechanism by which HCV causes HCC is not known. Unlike the hepatitis B virus (HBV), HCV is not a DNA virus and does not become incorporated within the nucleus of hepatocytes. It is more likely that HCC occurs against a background of inflammation and regeneration, associated with liver injury due to chronic hepatitis. Virtually all cases of HCV-related HCC occur in the presence of cirrhosis, suggesting that it is the underlying liver disease *per se* that is the risk factor for HCC rather than HCV infection [33] – although cirrhosis would not have occurred without the initial HCV infection. The prevailing hypothesis has been that some cirrhotic nodules that grow larger than others (referred to as adenomatous hyperplasia) were the precursor for HCC. Recently, however, it has been suggested that foci of transformed hepatocytes may arise in between cirrhotic nodules and grow to become adenomatous hyperplasia and, eventually, HCC. [34]

Host factors, which have been implicated in increasing the risk for development of HCC, are the same as for HCV progression: age, male gender, and severity of underlying liver disease. Viral genotype may be important, although early suggestions that infection with genotype 1b is more likely to result in the development of HCC have not been confirmed in larger studies. No clear link has been established between serum levels of HCV RNA and progression to HCC.

Some external factors that might add to the risk for HCC in patients with HCV infection include alcohol consumption, coexistent HBV infection, and porphyria cutanea tarda (PCT, an acquired or inherited photosensitivity with increased skin porphyrins, associated with iron loading and often with HCV) although this latter condition is found only in some geographic areas. [35] Other extrahepatic manifestations of HCV include non-Hodgkin's lymphoma, membranoproliferative glomerulonephritis, lichen planus, autoimmune thyroiditis, diabetes mellitus and Sjogren's Syndrome.

The risk for a patient with HCV infection developing HCC cannot be calculated with any precision. It is known that up to 20% of patients with chronic HCV infection develop histological evidence of cirrhosis over a 20-year period. Furthermore, among patients with established cirrhosis due to HCV infection in screening programs, it has been found that 3-4% per year develop HCC, at least for the first 4-5 years of screening. By extrapolation, after 20 years of infection, 6-8% of patients with chronic hepatitis C can be expected to have developed HCC, although these calculations need to be validated by more prospective studies. Studies from Japan have found that the mean interval from HCV mono-infection to the development of HCC is approximately 25 years, but these periods have a very wide range of variation. For reasons that are unclear, HCV outcomes seem to be worse in Japan than the US. In the United States, HCC has been described as soon as 5 years from the onset of HCV infection, although this is rare.

UK guidelines recommend that patients who are known to have cirrhosis or transition to cirrhosis should be considered for regular screening with biannual or more frequent ultrasound and alpha-fetoprotein (AFP) measurements to enable the early detection of hepatocellular carcinoma (HCC). It should be recognised that even with frequent screening a treatable HCC may not be detected. [12]

Typically, HCC carries a poor prognosis, with survival times from diagnosis measured in months. Screening studies have shown that small amounts of HCC can be detected at an early stage when it may be more amenable to curative therapy. At present surgical resection offers the best hope for prolonged disease-free survival. This may take the form of partial or total hepatectomy. Unfortunately, partial hepatectomy for HCC is associated with a very high recurrence rate (approximating 25% per year) while total hepatectomy implies liver transplantation. [36]

Conclusion

The above account of the postulated mechanisms by which HCV and HIV cause severe liver damage highlight targets for therapeutic intervention. These include direct inhibition of viral replication, immuno-modulation and anti-fibrotic measures, which will be discussed in the second part of this review article, together with clinical management of HIV/HCV coinfection.

Further reading: *British HIV Association (BHIVA) guidelines for treatment and management of HIV and Hepatitis C co-infection. October 2004.* Online at:

<http://www.bhiva.org>

Thanks to Tracy Swan and Daniel Raymond for editorial comments on this article.

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TREATMENT ACCESS

Forty injured, ten shot at peaceful protest to demand treatment in South Africa

Simon Collins, HIV i-Base

On 12 July a peaceful demonstration, organised by HIV-positive members of the Treatment Action Campaign in South Africa to ensure that people with HIV/AIDS receive antiretroviral treatment at Frontier Hospital and throughout the Eastern Cape, led to the South African Police Services in Queenstown brutally assaulting and then shooting unarmed, peaceful protesters asking for HIV treatment.

Forty people were injured and ten were treated for gunshot wounds. One person had to be admitted to hospital. At least ten of the injured people were people who live openly with HIV/AIDS. The majority of the protesters were women. At no stage was there violence, threat of violence or any form of provocation and the March had been planned with prior knowledge of local health authorities. No warning to disperse was issued as is required by law. After the assault, as people ran away, the police opened fire with firearms and then used teargas.

Basic demands from the demonstration included access to information on the number of people, tested, counselled along with information on successes and challenges of the treatment programme, which currently treats fewer than 200 or the 2000 local people estimated to need treatment. More people have died waiting for treatment than people on treatment.

Within 2 days of this news the international reaction quickly resulted in letters of protest from over 200 HIV support organisations in solidarity with the South African protestors.

For further details see the TAG website:

<http://www.tac.org.za>

Source: Treatment Action Campaign press release. 'Forty injured, ten shot at peaceful protest to demand treatment' 13 July 2005.

FDA approve additional Indian generics

Simon Collins, HIV i-Base

In the last issue of HTB we reported that six Indian manufactured generic ARVs received FDA tentative approval.

Further approvals have since been granted for a generic combination drug product consisting of lamivudine and zidovudine, manufactured by Aurobindo Pharma (on 7 July 2005); and zidovudine tablets manufactured by Ranbaxy Laboratories (on 13 July).

Tentative approval means that although existing patents and/or exclusivity prevent marketing of this product in the United States, it meets the quality, safety and efficacy standards for U.S. marketing. This makes these generic products eligible for purchase and use outside the United States under the President's Emergency Plan for AIDS Relief (PEPFAR).

When this fast-track approval for generic antiretroviral drugs was first announced there was wide skepticism that may have been a stalling mechanism in order to retain preferential purchase but the US government for US-manufactured brand ARV. While FDA approval shouldn't be necessary in addition to the WHO pre-qualification process, this rapid series of tentative approvals should at least end earlier attempts by some organisations to discredit the quality of the generic ARVs produced by the largest major Indian generic companies.

An archive of past list serve announcements is available on the FDA web site at:

<http://www.fda.gov/oashi/aids/listserve/archive.html>

Medical innovation and patent gridlock

John S. James, AIDS Treatment News

Is today's sheer multitude of biological patents (especially on genetics of human beings or human pathogens) killing medical innovation — in addition to generating prohibitive prices for vital medical care?

Today's pharmaceutical research and development has two huge problems, one widely recognized and the other often missed.

i) New-drug prices

Under the current system most of the world's people will have no access to new, patented drugs for up to 20 years. For example, India recently changed its pharmaceutical patent laws as required by the World Trade Organisation — and (at least initially) omitted steps the WTO allows to make treatments more available. Multinational and Indian corporations are clearly aiming to sell new drugs to the richest 5% to 10%, meaning that 90% of the entire population of India will be denied access. Around the world, a substantial majority of all human beings may be disqualified from new drugs by patents and the resulting monopoly pricing. Financial inequality is so great that companies can make more money by selling to a small elite at prices that only it can pay, than by selling to everybody.

A June 1, 2005, report in a major AIDS journal (*JAIDS*) from a study of 306 patients in India found that of those treated over a year with one of two 3-drug regimens available, 46% had lipodystrophy [1]. The researchers also found that lipodystrophy was significantly associated with d4T use, and called for "improving access to alternative less-offending drugs like tenofovir and abacavir." This article ominously documents the development of a new global system of second-class medical care, imposed by trade laws that countries throughout the world have been pressured to accept.

But the biggest train wreck may involve cancer, not AIDS. Increasingly there will be highly targeted, very effective drugs against specific cancers (although resistance does develop); most of them will be patented, priced at tens of thousands of dollars a year in the U.S. (and likely close to that in poor countries), and often needed by each patient indefinitely. Reportedly one cancer drug already had a price increase about ten times in India, after courts stopped a generic company from selling the less expensive version.

Medical care for the poor has always been a disaster. But in the past the main problem was lack of enough resources to go around. The modern problem is the worldwide imposition of a system designed to sell new treatments at artificially high monopoly prices that most people cannot pay. In the U.S. many prescriptions are unfilled among the 45,000,000 uninsured. And those who are insured may be unable to afford copays, or denied treatment to save money.

ii) Patent Gridlock

Patent restrictions can block or greatly slow research and development of better treatments — threatening the lives and health of everyone, even the richest, as no amount of money can quickly buy treatments and data that have never been created.

Patents do help innovation by providing incentives for investors to fund research and development (this necessary funding could be provided in other ways; for example, see "Medical Innovation Prize Fund: New Idea in Drug Development," in *AIDS Treatment News*, 20 June 2005). But patents also block innovation. A patent means that only one company in the world can develop an idea (absent corporate dealmaking, a very inefficient process that can delay medicines for years as lawyers and executives battle with other or just don't have time). Usually research ideas are simply dropped once patent issues come into view. Yet the company with the patent is unlikely to be the one with all the other patents needed, with the interest in pursuing the project, and with the best technical, financial, and human resources (including the nebulous but all-important personal chemistry and enthusiasm) to create a new treatment successfully. Therefore the best research and development that could be done is unlikely to happen at all. Patent gridlock could be the major, overlooked reason for the unexpected big drop in the ability of pharmaceutical companies to produce truly innovative new medicines, despite huge advances in basic biological knowledge and in research tools.

Several years ago an official at a major pharmaceutical corporation said that it could not research about 100 potential cancer treatments its scientists wanted to test, because it could not obtain the rights at all, or could not negotiate an affordable price. If one of the world's biggest companies could not handle this problem in its own central field of work, think of the obstacles others must face.

Price negotiation always includes the risk of no agreement, as parties dream big and then walk away if they don't get what they want. But medical patents can burn the future as well as the present, escalating a failed negotiation into a legal blockade to critical research and innovation everywhere in the world — with life-and-death consequences for many who were never at the table.

Patent Gridlock — A Perverse Economics of Scale?

Economists should examine the possibility that a patent system with universal reach may become much less useful and more harmful when its universe gets too large — that gridlock develops because there are far more research projects that can be

blocked by patents or forced into less productive channels, and far more patents and claims in existence to block them. The U.S. patent system may have worked fairly well until about mid 20th century; then large companies increasingly used patents for blocking, getting hundreds of patents and thousands of claims to keep rivals out of an area entirely even if they do not infringe, just by the risk and cost of litigation. The “golden age” of drug development about 50 years ago may have been a legacy of the previous environment before gridlock set in — a small taste of what could have been possible with modern science, if it had not been choked off by a new golden age of overgrowth of patent rights and litigation.

Now that biological and pharmaceutical patents are enforced worldwide, have greatly increased in number, and have far more ongoing research to block, their damage to innovation may be much greater than their benefit. But this problem tends to remain invisible, because those involved are usually pledged to secrecy, and also are trying to work something out so that their projects can proceed at all, however unsatisfactorily.

Patents on the genetics of viruses or other naturally occurring disease mechanisms can be particularly destructive in stopping research on new treatments, as happened recently with hepatitis C. Ownership of pieces of the heredity of human beings or human diseases creates barriers to entry that are infinite, by act of Congress or other government bodies. Tens of thousands of these pieces of exclusive ownership of nature (sometimes controlling whole human diseases) may have created a fatal gridlock that is blocking the translation of new knowledge into practical medical treatments.

If this analysis is correct, pharmaceutical and related patents could be killing more people than all the wars on Earth combined, by blocking the research and development of new medical treatments for diseases that kill far more people than wars. This problem sneaked up on the world, mainly because of a change in scale — the sheer amount of both research and patent activity today, and their global reach, which dumps all patents into a single huge, universal pool, where each one can stop innovations anywhere. This possibility urgently needs expert and public attention.

Reducing Patent Gridlock

A June 2005 decision by the U.S. Supreme Court [2] should reduce patent gridlock in certain cases. The court unanimously allowed patented drugs to be used in preclinical studies to develop new drugs (but not for basic scientific research) without permission of the patent holder. The court said that its decision did not address whether research tools could be used similarly. Surprisingly, large pharmaceutical companies tended to side with consumer groups like AARP to support this decision, while smaller biotech companies as well as research universities tended to be opposed (due to legitimate fears that big pharma might not need to pay them for using their work in developing research tools, which will never generate revenue by being sold to patients as drugs).

This unanimous Supreme Court decision seems to open the door to well-crafted legislation to further relieve patent gridlock, while also allowing the development of research tools to be profitable. For example, a compulsory-licensing system could create a medical patent pool, allowing big pharma, universities, or anyone else doing medical research to make free use of a broad class of patents, in return for a royalty based on sales, when and if any products developed with those patents were sold. Then any company or other organization could use almost any technology for medical research, without the hassle, expense, and delay of patent negotiation. This would free the research process, make royalties more predictable, and pay for innovation at the time of product sales — after the work has already proven itself, and when a revenue stream exists. Paying for rights as a percentage of sales (instead of as a large fixed cost) could also encourage low prices in poor countries — distributing research costs more equitably and realistically.

Open-source projects could also conduct rights-free medical research and drug development under the same rules. If they never made sales they would never owe royalties on the patents they used. And their work could greatly increase the value of patents by finding and proving new uses for them, at no cost to their owners.

A less ambitious approach to open-source type development in pharmaceuticals (not suggesting any legal changes) was discussed last year in *The Economist*, generally favorably (“An Open-Source Shot in the Arm?” June 10, 2004). Here the idea was to move the collaboration that already works well in bioinformatics (such as in the human genome project, a clear example of successful sharing of data) further downstream, closer to the patient. For example, effective worldwide collaboration and data sharing might greatly increase the discovery of new uses for off-patent or otherwise unpatentable drugs. (Recently the common antibiotic doxycycline was found to be a major advance for treating filariasis [elephantiasis], one of the most common causes of disability worldwide — by killing bacteria needed by the worms that cause the disease, leading to their eventual death. [3]) Online research projects could also organize volunteer and other contributions toward developing entirely new drugs for tropical and other neglected diseases (see the Tropical Disease Initiative, <http://www.tropicaldisease.org>)

An important article on Internet collaboration (“The Power of Us,” *Business Week*, June 20, 2005, page 75-82) describes many examples of successful new business projects based on the work of many people throughout the world, often volunteers, made possible by widespread online communication. Open-source software development is just one of these.

This approach will surely be different in pharmaceuticals than in software, because the industries are so different. It may contribute greatly to some projects, by allowing research to proceed at full speed, without unworkable rights constraints. This is already happening, but only in a few areas so far, where some way can be found around the prevailing patent gridlock.

The current system of drug development is failing. The time for exploring new ideas and practices is now.

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<http://www.aidsnews.org>

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ANTIRETROVIRALS

US adult guidelines updated - July 2005

The Panel on Clinical Practices for Treatment of HIV Infection is providing the readers with the following supplemental recommendations to the April 7, 2005 Guidelines for the Use of Antiretroviral Agents in HIV-1 Infected Adults and Adolescents. Please note that these recommendations are in effect immediately. The text and tables of the full document will be updated at a later date.

Below are the supplemental recommendations:

1. The Panel recommends that a regimen containing “tenofovir + didanosine + NNRTI” should not be used as an initial regimen in antiretroviral treatment naïve patients.

This recommendation is based on results from several small observational studies and pilot clinical trials showing a high rate of early virologic failure in treatment-naïve patients who received this combination as their initial regimen. Emergence of resistant mutations to NNRTIs and to tenofovir and/or didanosine (K65R or L74V mutations) was frequently seen in patients who failed to respond to this combination. [1-4]

Of note, patients with high baseline HIV-RNA (> 100,000 copies/mL) and low CD4+ T-cell counts (< 200 cells/mm³) were particularly at risk of early virologic failure. There are not enough data for the combination of tenofovir/didanosine with protease inhibitor in treatment-naïve patients to assess virologic responses of this regimen, thus, there is no recommendation for or against the use of this combination at this time.

2. The Panel recommends that lopinavir/ritonavir can be dosed as one single daily dose (6 capsules or 10 mL - equivalent to 800mg lopinavir/200mg ritonavir) in treatment-naïve patients.

Once daily dosing is not recommended in treatment-experienced patients or in patients receiving concomitant efavirenz, nevirapine, amprenavir (or fosamprenavir), or nelfinavir.

This recommendation is based on 48-week data from two clinical trials comparing once vs. twice daily lopinavir/ritonavir, used in combination with tenofovir + emtricitabine in treatment-naïve patients, demonstrating similar virologic responses in both treatment arms.

Once daily dosing has not been studied in treatment-experienced patients. In the pharmacokinetic study, the trough concentration of lopinavir at the end of a 24 hour dosing interval was found to be approximately 60% lower than with twice daily dosing. Given the lower trough concentration and no clinical trial data in treatment-experienced patients, once daily dosing is currently not recommended in these patients.

It is also important to note that moderate to severe diarrhea were reported significantly more frequently in subjects who received once daily lopinavir/ritonavir as compared to twice daily dosing (16% vs. 5% respectively). [5]

Source: FDA listserve announcement, July 2005

An archive of past list serve announcements is available on the FDA web site at:

<http://www.fda.gov/oashi/aids/listserve/archive.html>

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FDA filing of T-20 sNDA for administration with a needle-free injection device

Roche and Trimeris announced on 18 July 2005 that the U.S. Food and Drug Administration (FDA) has accepted the filing of their sNDA to consider inclusion of information about the Biojector 2000 (B2000) needle-free injection device in the T-20 (enfuvirtide, Fuzeon) labeling. The filing is based on data from the T20-405 study, a single-dose, comparative pharmacokinetic study of T-20 administered via the needle-free device compared to standard needle-syringe administration. Results from this study were reported in the April 2005 issue of *HIV Treatment Bulletin*. [1]

The B2000 injection system, manufactured by Bioject Medical Technologies Inc., is a needlefree CO₂-powered injector that disperses liquid medication through the skin. The B2000 has been available since 1996 to deliver subcutaneous and intramuscular injections and has been used in vaccine delivery, chronic therapy and other settings, delivering millions of injections.

Source: Roche Press Release

For more information on Biojector:

<http://www.bioject.com>

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1. Needle-free injections for T-20 in US. *HTB* April 2005.
<http://www.i-base.info/htb/v6/htb6-4/Needle.html>

OTHER NEWS

UK kidney transplant guidelines online

These new guidelines, written by Dr Sanjay Bhagani and Dr Paul Sweny on behalf of the British HIV Association (BHIVA), and reviewed and endorsed by the British Transplantation Society Standards Committee, are now available to view online, and to download as Word and pdf documents, from the BHIVA website:

<http://www.bhiva.org>

New study urges caution over widespread criminalisation of HIV transmission

A new report by the Global Network of People living with HIV/AIDS Europe (GNP+ Europe) and Terrence Higgins Trust highlights the widespread criminalisation of HIV transmission across Europe and calls for an informed and measured approach based on public health and human rights.

The UNAIDS funded report, *Criminalisation of HIV transmission in Europe*, comes at a critical time, amid media hype surrounding HIV transmission cases in the UK, the Netherlands, Sweden and Finland.

The report identifies and analyses the laws used in relation to HIV transmission and maps prosecution within signatory States of the European Convention of Human Rights. It also discusses the value and appropriateness of the use of criminal law and other punitive measures in the response to the epidemic.

Until recently the majority opinion seemed to be that criminal law should only be used in the context of HIV as a last resort, for example in cases of rape or wilful deception.

Many different types of law are used to prosecute transmission of HIV, including HIV-specific laws and general criminal law provisions. Some laws require intent, some do not. Some laws criminalise only actual transmission, while others criminalise the risk of transmission. Furthermore, some laws include "reckless" as well as "negligent" behaviour in addition to "intentional" behaviour in their legal provisions.

Though data on the background of people prosecuted was hard to find, it appears that a substantial number are from marginalised groups, in particular migrants. Men appear more likely to be prosecuted than women and there have been no traceable convictions for transmission from mother to baby.

Lisa Power, Head of Policy at Terrence Higgins Trust said: "We urge lawmakers to take an informed approach based on human rights and public health if they wish to bring the law to bear on HIV transmission. "Criminalising consensual sexual acts will discourage people with HIV from seeking help in maintaining safer sex and drive such behaviour underground. Positive support to maintain safer sex is a basic part of preventing onward transmission."

The full report, 'Criminalisation of HIV transmission in Europe' can be downloaded at:
<http://www.gnpplus.net> and
<http://www.tht.org.uk>

Canada changes visa process for HIV-positive visitors – an example for the US?

As a result of ongoing discussions between government departments, the organisers of the XVI International AIDS Conference (AIDS2006) due to be held in Toronto in August 2006, and others, Canada no longer requires people applying for visas as short-term visitors to disclose their HIV status on the application form.

Previous immigration policy

Canadian immigration law provided that a person may be denied a visa or entry to the country as "medically inadmissible" if:
a) they are "likely to be a danger to public health or public safety"; or b) they "might reasonably be expected to cause excessive demand on health or social services" - and specifically, if they would add to waiting lists for services and thereby add to morbidity or mortality as a result of denial or delay of these services for Canadian citizens or permanent residents.

Generally, neither of these grounds applies to a person living with HIV/AIDS seeking to enter the country as a visitor on a short-term basis (i.e., under 6 months).

- HIV is not a casually communicable infectious disease (unlike tuberculosis). It is Canadian government policy that people living with HIV/AIDS do not represent a danger to public health or safety by virtue of their HIV status.
- Similarly, Canadian policy states that a person living with HIV/AIDS entering the country on a short-term basis "would not normally be expected to place a demand on health services".

Canada has now amended its application form for a "temporary resident visa" to change the health-related questions posed to visa applicants. In May 2005, the new visa application form was implemented by CIC.

As a result of the recent change, Canada does not require people applying for a visa to enter Canada as a short-term visitor to disclose known HIV infection on the visa application form.

For more information: Canadian HIV/AIDS Legal Network's website
<http://www.aidslaw.ca/Maincontent/issues/Immigration>

Source: gender-aids@forums.healthdev.org

C O M M E N T

This positive approach to allow HIV-positive individuals the right to travel could easily be adopted by the USA which still maintains a discriminatory policy that includes HIV as a barrier to enter the country. This is why IAS conferences are no longer held in the USA.

Current US policy does not include either visiting family or vacation as a reason to grant a visa for an HIV-positive person. Advice for HIV-positive individuals to apply for a visa for these reasons will not help them visit the US.

ON THE WEB

Medscape articles and online papers:

The following journal articles are available online in full. Medscape requires a simple one-time free online registration.

Journal of AIDS (JAIDS):

http://www.medscape.com/viewpublication/878_index

- **Incidence of pancreatitis in HIV-1-infected individuals enrolled in 20 AACTG studies: lessons learned**
- **Intermittent low-level viremia in very early primary HIV-1 infection**

HIV Medicine:

http://www.medscape.com/viewpublication/1008_index

- **The use of a triple nucleoside-nucleotide regimen (tenofovir+d4T+3TC) for non-occupational HIV Post-Exposure Prophylaxis (nPEP)**
- **Osteoprotegerin and bone turnover markers in heavily pretreated HIV-Infected patients**

The AIDS Reader:

http://www.medscape.com/viewpublication/93_index

- **Spectrum of human papillomavirus-related dysplasia and carcinoma of the anus in HIV-infected patients**
- **A diagnostic dilemma in a patient receiving antiretroviral and antituberculosis therapy**

Community reports and journals:

FCHR report on monitoring technologies for resource-limited settings

This report from a workshop organised by the Forum for Collaborative HIV Research on February 26th 2005 is now available online:

<http://hivforum.org/uploads/CROI%202005/Feb%2026%20full%20day%20report.pdf>

Treatment Action Group report on HIV, HCV, TB treatment and vaccine pipeline

<http://www.aidsinfonyc.org/tag/>

Two new documents are now available from TAG.

- **Pipeline Report - July 2005**

What's in the Pipeline: New HIV Drugs, Vaccines, Microbicides, HCV and TB Treatments in Clinical Trials

- **Antiretrovirals pipeline updated - June 2005**

Updated to include links to CROI 2005 abstracts.

MEETING ANNOUNCEMENTS

3rd Advanced HIV course – EACS

29-31 August 2005, Montpellier, France

The European AIDS Clinical Society (EACS) are running their third course on 'Antiretroviral therapy and comprehensive care' focused on the clinical management on HIV.

Tuition fee is 100 euros and includes accommodation for four nights, lunches for the three-day course and course material.

Please contact the EACS office for details:

sylvie-chatelain@eacs.ws

<http://www.eacs.ws>

International Association of Physicians in AIDS Care

European Sessions, 6-7 October 2005

Co-Chairs: Bernard Hirschel, MD; Joep MA Lange, MD, PhD

This meeting is intended for HIV-treating physicians and is limited to 100 delegates. Registration is on a first-come first-served basis. Please post and share this information with physicians in your department.

If you are a member of IAPAC, please note that there is a registration fee of \$100 USD. If you are not a member, your fee is US\$200 and will include a one-year membership to IAPAC.

For more information and registration please contact Carrie Scharrer at:
cscharrer@iapac.org

or call +1 312 795 4935 (U.S.contact number)

or visit our website (and click on the IAPAC European Sessions 2005 banner) at:
<http://www.iapac.org/>

We hope you will be interested in attending Sessions and look forward to your participation.

PUBLICATIONS AND SERVICES FROM i-BASE

i-Base website redesigned

<http://www.i-Base.info>

The website has been totally redesigned so that it is faster and easier to use, and is more accessible for those with impaired sight. For those who understand these matters, all pages conform to at least the W3C-WAI Level A and most to level AAA.

All i-Base publications are available on this website, including 2005 editions of four treatment guides. The site gives details about i-Base, the UK Community Advisory Board (UK CAB) and World CAB, our phone service and meetings, as well as access to our archives and an extensive range of links. It can be used to order publications and arrange for regular subscriptions to be delivered by post or email (as PDF files).

A new section on education and training includes a training manual with eight 2-hour modules that include questions and evaluation. Subjects start from the basics including CD4, viral load and other monitoring tests, combination therapy and side effects, to brief overviews of the main opportunistic infections. There is a module on pregnancy and another module on IV drug users and treatment

UK CAB: reports and presentations

The UK Community Advisory Board (UK CAB) is a network for community treatment workers across the UK that has been meeting for three years. Each meeting includes two training lectures and a meeting with a pharmaceutical company or specialist researcher.

Reading material, reports and presentations from these meetings (the 14th meeting is on 19 August this year) are posted to the i-Base website and are available in printed format.

The August meeting will include reports back from the IAS conference and a meeting with Roche about European and UK access to the Biojector needle-free delivery system for T-20.

<http://www.i-base.info/ukcab/index.html>

Reports on international drug pricing

Two reports from meetings between community advocates and pharmaceutical companies, that focussed on pricing issues and global access to treatment, and that are now available online.

The latest report focusses on a meeting held in January 2005 with four Indian generic manufacturers.

An earlier report is from a meeting in February 2004 with three major brand manufacturers.

Both are available to download as a pdf file from the i-Base website.

<http://www.i-base.info/wcab/index.html>

Introduction to Combination Therapy

June 2004 edition

The booklet explains what combination therapy is, how well it works, who can benefit from it, when to start taking it, some differences between treating men and women, side effects, the best combinations, changing treatment, taking part in drug trials, your relationship with your doctor, the importance of adherence, and how to avoid drug resistance.

Guide to HIV, pregnancy & women's health

New Spring 2005 edition

Updated and revised in April 2005, this patient guide helps women get the most out of HIV treatment and care before, during and after pregnancy. It should help whether you are on therapy or not and includes information for your own health and for the health of your baby.

Guide to changing treatment: what to do when your treatment fails

New April 2005 edition

Also updated and revised in April 2005, this is a non-technical patient guide to changing treatment and what to do if your treatment fails.

This booklet helps patients in discussions with doctors, and covers what you can do if your viral load starts to rise, and the importance of considering or finding out why your current combination failed, treatment strategies and new and pipeline treatments.

Guide to avoiding & managing side effects

New February 2005 edition

This is a comprehensive 44-page guide that is aimed at helping anyone using HIV drugs to get the most out of their treatment, the most out of their relationships with their doctor and other health professionals, to get better medical care to improve their health and, most importantly, to enjoy a better quality of life.

New sections are included on heart disease, lipodystrophy, and information relating to newer drugs including T-20, atazanavir, tenofovir, FTC and fosamprenavir.

Treatment 'Passports'

These popular booklets are for HIV-positive people – whether newly diagnosed or positive for a long time - to keep a record of health and treatment history. Like all i-Base publications, they are available free as single copies, or in bulk.

HIV Treatment Bulletin (HTB)

This is the journal you are reading now: a review of the latest research and other news in the field. HTB is published 10 times a year in a printed version, in a pdf file that we can email to you, and on our website. All subscriptions are free.

Treatment information request service – 0808 800 6013

i-Base offers specialised treatment information for individuals, based on the latest research.

We provide information over the phone, and we can mail or email copies of the latest research studies relevant to the caller.

For details, call the i-Base treatment information free phone line on 0808 800 6013. The line is usually staffed by positive people and is open Mondays, Tuesdays and Wednesdays from 12 noon to 4pm. All calls are in confidence and are free within the UK.

Find HTB on AEGiS

AEGiS.org - the longest established and largest global resource of online HIV information - includes HTB in the regular journals that it puts online. You can find us at:

<http://www.aegis.org/pubs/i-base/2004>

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h-tb

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Editor in Chief: Paul Blanchard

Editor: Simon Collins

Commissioning Editor: Polly Clayden

Medical Consultants:

Dr Sanjay Bhagani, Royal Free Hospital, London.

Dr Karen Beckerman, Bellevue Hospital, New York.

Dr Gareth Hardy, Royal Free Hospital, London.

Dr Saye Khoo, University of Liverpool Hospital.

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HIV i-Base
Third Floor East
Thrale House
44-46 Southwark Street
London SE1 1UN
T: +44 (0) 20 7407 8488
F: +44 (0) 20 7407 8489

<http://www.i-base.info>

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