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AIDS 2008, **22**:993–998

Determination of a major histocompatibility complex class I restricting simian immunodeficiency virus $Gag_{241-249}$ epitope

Several major histocompatibility complex class I (MHC-I) alleles such as *HLA-B*57* have been shown to be associated with lower viral loads and better prognosis in HIV-1 infections, and MHC-I-restricted epitope-specific effective cytotoxic T lymphocyte (CTL) responses are found to play an important role in this reduction of viral loads [1–3]. Characterization of these effective CTLs could contribute to the development of an effective AIDS vaccine.

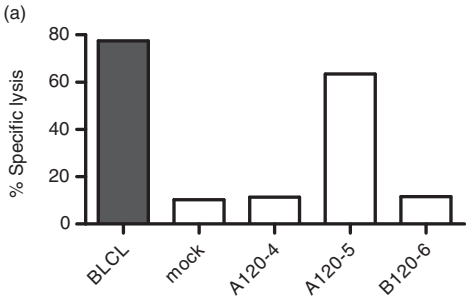
We have developed a prophylactic vaccine using a Sendai virus vector expressing simian immunodeficiency virus mac239 (SIVmac239) Gag (SeV-Gag) and have shown its protective efficacy against SIVmac239 challenge in a group of Burmese rhesus macaques (*Macaca mulatta*) sharing an MHC-I haplotype *90-120-Ia* [4]. Involvement of SIVmac239 $Gag_{241-249}$ (SSVDEQIQW) epitope-specific CTL responses in this viral control have been indicated [5]. Interestingly, the SIVmac239 $Gag_{241-249}$ epitope is located in a region corresponding to the HLA-B*57-restricted HIV-1 $Gag_{240-249}$ epitope, TW10 (TSTLQEQIAW), and TW10-specific CTL responses have also been indicated to exert strong suppressive pressure on HIV-1 replication resulting in lower viral loads [6,7]. An SIVmac239 $Gag_{241-249}$-specific CTL escape mutation has been shown to result in a loss of viral fitness similarly with a TW10-specific CTL escape mutation [5]. In the present study, for further analysis of SIVmac239 $Gag_{241-249}$-specific CTL function, we have tried to determine the MHC-I that restricts this CTL epitope.

Among eight MHC-I alleles consisting of MHC-I haplotype *90-120-Ia* [4,8], expression of three alleles, *Mamu-A*90120-4, Mamu-A*90120-5*, and *Mamu-B*90120-6*, was predominant at RNA levels. We cloned cDNAs of these three alleles and established HLA-A/B/C-negative human 721.221 cell lines [9] expressing these cDNAs, respectively. These cells were pulsed with 10 nmol/l of $Gag_{241-249}$ peptide and used as target cells for the CTL assay using an SIVmac239 $Gag_{241-249}$-specific CTL clone as the effector. Measurement of cytotoxicity in standard ^{51}Cr release assay [5] revealed specific killing of $Gag_{241-249}$-pulsed cells expressing Mamu-A*90120-5, indicating restriction of this CTL epitope by the Mamu-A*90120-5 molecule (Fig. 1a).

Both of the Mamu-A*90120-5-restricted SIVmac239 $Gag_{241-249}$ epitope and the HLA-B*57-restricted HIV-1 TW10 epitope are considered to have the same anchor residues, serine (S) at position 2 and tryptophan (W) at the carboxyl terminus. Comparison of amino acid sequences of antigenic peptide-binding domains ($\alpha1$ and $\alpha2$ domains) in Mamu-A*90120-5 with those in HLA-B*5701 revealed limited similarities (154/182 = 84.6%) between these two (Fig. 1b). This might be compatible with previous reports indicating that human and macaque MHC-I molecules with divergent peptide-binding grooves can bind similar or identical peptides [10,11]. MHC-I molecules form a peptide-binding groove including B-pocket and F-pocket that play a key role in determination of the binding peptide motif for its specific binding to the MHC-I. Mamu-A*90120-5 and HLA-B*5701 showed similarity in eight of 11 residues at 7, 9, 24, 25, 34, 45, 63, 66, 67, 70, and 99, which are considered to be anchor residues involved in B-pocket binding and in seven of eight residues at 77, 80, 81, 116, 123, 143, 146, and 147 involved in F-pocket binding [11–13].

In addition, TW10 epitope-specific CTLs, HLA-B*57-restricted HIV-1 $Gag_{147-155}$ [ISW9 (ISPRTLNAW)] epitope-specific CTLs have also been indicated to exert strong selective pressure on HIV-1 [14]. The SIVmac239 $Gag_{149-157}$ amino acid sequence corresponding to the HIV-1 $Gag_{147-155}$ epitope region is LSPRTLNAW, showing a difference at the amino terminus, and CTL responses specific for a peptide including the SIVmac239 $Gag_{149-157}$ amino acid sequence were not induced by SeV-Gag vaccination in Mamu-A*90120-5-positive macaques (data not shown). Interestingly, the SIVmac239 Gag 148th proline (P) and 149th leucine (L) correspond to the HIV-1 Gag 146th P and the 147th L, respectively that have been indicated to be selected in HIV-1-infected humans possessing HLA-B*57. Selection of the former 146th P has been shown to result in escape from ISW9-specific CTL recognition by disturbance in antigen processing [14]. Thus, it is speculated that the SIVmac239 $Gag_{149-157}$-derived peptide may not be presented by Mamu-A*90120-5 even if it has an ability to bind this peptide.

Both SIVmac239 $Gag_{241-249}$-specific CTLs and HIV-1 TW10-specific CTLs have been indicated to exert strong suppressive pressure on SIV/HIV-1 replication and select for a mutation resulting in escape from their recognition at the cost of viral fitness. Thus, this Gag region may be a promising CTL target for viral control, and SIVmac239 infection in Mamu-A*90120-5-positive macaques could be a unique model for examining viral replication in the



(b)

```
                  * *                 **          *
Mamu-A*90120-5  GSHSMRYFYT SMSRPGRGQP RFFAVGYVDD TQFVRFDSDA
HLA-B*5701      ---------- A-------E- --I------- ----------

                    *                   *  **  *       #  #
Mamu-A*90120-5  ESPRMEPRAP WVEQEGPEYW DQETRKAKDT AQNYRVNLRT
HLA-B*5701      A----A---- -I-------- -G---NM-AS --T--E---I

                #                   *                   #
Mamu-A*90120-5  ALRYYNQSEA GSHTIQKMYG CDLGPDGRLL RGYDQSAYDG
HLA-B*5701      ---------- ---I--V--- --V------- --H-------

                  #                   #  ##
Mamu-A*90120-5  RDYIALNEDL RSWTAADMAA QNTQRKWEAA GWTEQMRAYL
HLA-B*5701      K--------- S------T-- -I-------- RVA--L----


Mamu-A*90120-5  EGECLEWLRR YLENGKETLQ RA
HLA-B*5701      --L-V----- ---------- --
```

Fig. 1. Mamu-A*90120-5 that restricts the SIV $Gag_{241-249}$ epitope. (a) CTL assay using a $Gag_{241-249}$-specific CTL clone on a B-lymphoblastoid cell line derived from a macaque possessing *90-120-Ia* (BLCL), 721.221 cells (mock), and 721.221 cells expressing Mamu-A*90120-4 (A120-4), Mamu-A*90120-5 (A120-5), and Mamu-B*90120-6 (B120-6), respectively. (b) Amino acid sequences of the Mamu-A*90120-5 α1 and α2 domains in comparison with HLA-B*5701. The anchor residues involved in B and F-pocket binding are indicated by * and #, respectively.

presence of those CTLs targeting this region like TW10-specific CTLs. Finally, we obtained a phycoerythrin-conjugated $Gag_{241-249}$ epitope-Mamu-A*90120-5 tetramer for specific detection of $Gag_{241-249}$-specific CTLs. This could be useful for the analysis of $Gag_{241-249}$-specific CTL responses in Mamu-A*90120-5-positive macaques infected with SIVmac239.

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Does tenofovir increase efavirenz hepatotoxicity?

Antiretroviral drugs have the potential to cause liver toxicity, especially in hepatitis B virus or hepatitis C virus coinfected patients. Tenofovir is among the few antiretrovirals that are considered nonhepatotoxic, whereas efavirenz can cause liver enzyme elevations [1,2]. We report three cases of liver enzyme elevations in persistently hepatitis B virus and hepatitis C virus-negative, HIV-infected patients after the addition of tenofovir to an efavirenz-containing regimen.

Patient 1

A 58-year-old Caucasian man was on virologically successful antiretroviral therapy (zidovudine, lamivudine and efavirenz, respectively) since July 2002. In July 2007, zidovudine was replaced by tenofovir because of lipoatrophy and bone marrow toxicity. Four weeks later, alanine aminotransferase (ALT, normal values <50 IU/l) was 92 IU/l and aspartate aminotransferase (AST, normal values <50 IU/l) was 62 IU/l. Both enzymes had always been within the normal range prior to the switch. Further controls showed ALT 144 IU/l and AST 84 IU/l (after a further 1 month) and ALT 142 IU/l and AST 77 IU/l 3 months after tenofovir introduction. The patient then stopped tenofovir and began didanosine. Three weeks later, ALT was 48 IU/l and AST was 44 IU/l.

Patient 2

A 34-year-old African woman was on zidovudine, lamivudine and abacavir since September 2003. In October 2006, abacavir was replaced by efavirenz because of virological failure. In August 2007, owing to anaemia, zidovudine was stopped and tenofovir was started. In September 2007, ALT and AST (previously normal) were 133 and 199 IU/l, respectively; liver enzyme elevation was confirmed subsequently after 3 weeks (ALT 186 IU/l, AST 146 IU/l). Highly active antiretroviral therapy (HAART) was stopped and, in the beginning of November 2007, ALT and AST were back to normal (36 and 30 IU/l, respectively). The patient is on abacavir, lamivudine and lopinavir/ritonavir since December 2007.

Patient 3

A 30-year-old Caucasian man was on lamivudine, tenofovir and efavirenz since April 2007. In May 2007, ALT and AST (previously normal) were 392 and 225 IU/l,. ART was discontinued and, 40 days later, ALT was 23 IU/l and AST was 29 IU/l, respectively. The patient is on didanosine, lamivudine and nevirapine since December 2007.

No cases of tenofovir-related hepatotoxicity have been reported in the literature, and the drug appears to be well tolerated even in cirrhotic patients [1]. In contrast, numerous cases of hepatotoxicity are related to efavirenz use [2,3]. Interestingly, in individuals who are slow efavirenz metabolisers, such as those with CYP2B6 loss/diminished-function alleles, efavirenz plasma area under the curve values are highest among patients receiving tenofovir [4], and an unexpected development of neuropsychiatric adverse events has been reported following addition of tenofovir to an efavirenz-containing ART regimen [5]. We have not measured efavirenz plasma concentrations in our three patients, and therefore we cannot prove whether an increased efavirenz plasma concentration is responsible for the observed rise in aminotransferase levels. Alternatively, hepatotoxicity may be responsible for a highly infrequent tenofovir-related side-effect. Analysis of large databases or pharmacokinetic studies is needed to confirm, extend and explain our observations.

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Methodological issues of non-inferiority trials in HIV-infected patients: a need for consensus?

We read with great interest the publication by Pulido *et al.* [1] who reported the results of a randomized trial evaluating a lopinavir–ritonavir monotherapy for maintenance in HIV-infected patients. On the basis of the primary endpoint, the authors concluded that maintenance with lopinavir–ritonavir monotherapy is non-inferior to a triple therapy in the studied population. The authors also acknowledged the limitations of their results, in particular the fact that non-inferiority was not demonstrated for all secondary endpoints.

The present report illustrates some of the methodological difficulties in the design and analysis of non-inferiority trials for HIV treatment strategies in general.

First, we are concerned by the apparent absence of a consensus regarding the choice of the primary endpoint in trials comparing different strategies of antiretroviral treatment. Pulido *et al.* [1] chose a composite endpoint to define therapeutic failure as follows: confirmed HIV RNA higher than 500 copies/ml, or loss to follow-up, or treatment discontinuation, or change of randomized therapy other than reinduction. According to the provided definition, cases in the monotherapy group with confirmed virological failure (two measurements of HIV RNA $>$ 500 copies/ml separated by at least 2 weeks) are not considered failures, if HIV RNA is resuppressed successfully after reindroduction of nucleosides. To our knowledge, this is an uncommon choice as compared with the endpoints of other randomized trials evaluating simplification regimens in HIV-infected patients [2–4]. Yet, if reinduction in the monotherapy group is not considered as therapeutic failure, non-inferiority of the two treatment strategies is more likely to be demonstrated. For example, one could assume that due to early virological failure, a number of the patients would receive reinduction treatment shortly after the switch to monotherapy. Consequently, these patients would receive the same treatment as the comparator group for almost the entire length of the trial, which in turn would downsize the difference between the two groups over the time of the trial. Thus, we believe that the secondary analyses reported by the authors, in which treatment modification was considered as failure, constitute a more cautious choice. In that case, the authors could not conclude consistently that the simplification strategy was non-inferior to a triple therapy in the studied population.

Second, we suggest that some aspects concerning the treatment of missing data and the statistical approach in non-inferiority trials should be further clarified. In some of the analyses reported by Pulido *et al.* [1], the authors considered missing data to be failures. It is noteworthy that this approach tends to equalize outcomes in the compared groups. This effect is deliberate in superiority trials, but it may be inappropriate in non-inferiority analyses as it minimizes the difference between groups [5]. Pulido and colleagues thus tested the robustness of their results by performing an as-treated analysis. The results of additional sensitivity analyses would be more convincing by using the worst-case methodology to quantify the potential for bias due to missing data, that is considering missing data to be failures in the intervention group, but successes in the comparator group and vice versa. Indeed, a per-protocol (or as-treated) analysis in non-inferiority and equivalence designs might also bias the results towards a smaller difference between groups [5–7]. The worst-case method, by contrast, may provide a truly conservative assessment of the robustness of a binary endpoint in a non-inferiority trial and its broader application should be discussed for future trials.

Third, there is a need for a large consensus regarding the non-inferiority margin in trials evaluating maintenance strategies in treatment-experienced patients with suppressed HIV replication. For an assumed failure rate of 10%, Pulido *et al.* [1] defined a non-inferiority margin of 12%, without commenting on the latter choice. The lack of rationale for the non-inferiority margin seems indeed common in HIV trials [8], and its relevance remains to be properly assessed. According to the authors' premise, a failure rate of up to 22% is accepted in pretreated patients in whom viral replication is controlled prior to randomization. We postulate that the acceptability of this assumption should be scrutinized [9]. A consensual, clinically relevant non-inferiority margin should be defined for a given response rate and be applied to all non-inferiority trials in this population, as has been proposed in other research areas [10].

In summary, some key aspects of non-inferiority trials in HIV-infected patients warrant thorough methodological deliberation. We need an international consensus to help design future non-inferiority trials in HIV patients, as these trials are more and more common, given the potency of current antiretroviral drugs.

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Incidence of pancreatitis in HIV-infected patients, and the association with antiretroviral therapy

We recently reported a low incidence of pancreatitis in a European cohort of HIV-positive individuals followed prospectively from 2001 to 2006, with a rate of 1.27 cases per 1000 person-years [1]. Fessel and Hurley [2], in an editorial comment in the same issue of *AIDS*, reported a much higher incidence of approximately five times that seen in our study in the years 1996–2006 in a North American cohort. The authors noted that the rate of pancreatitis remained constant over time. However, we feel that there are important differences in the definition of pancreatitis used in the two studies that may go some way to explaining the disparate incidence rates observed.

Fessel and Hurley use a definition of pancreatitis based on either the presence of plasma lipase greater than four times the upper limit of normal (ULN), amylase greater than six times the ULN, or a pancreatitis diagnosis captured in the electronic medical record. In contrast, the EuroSIDA study used a detailed case definition of pancreatitis, and all events were source verified, reviewed, and classified centrally by the study physicians. Even when considering presumptive pancreatitis, two of the following three events were required: one or more characteristic symptoms or characteristic signs of pancreatitis; raised enzymes; at least one imaging investigation suggesting pancreatitis according to a radiologist or clinician. Furthermore, raised amylases were only considered as a pancreatitis event if other aetiology could be excluded. Only if definitive source documentation could not be obtained was a pancreatitis event assumed without further investigation. Thus, we suggest that the EuroSIDA study group used more stringent criteria to define pancreatitis events that included exclusion of other possible causes of abnormal laboratory values, and required the presence of clinical manifestation of disease in nearly all cases. Thus, a lower incidence of pancreatitis would be expected in our study when compared with that found when using the definition employed by Fessel and Hurley.

Additionally, the authors highlight the lack of an association between pancreatitis and the use of stavudine and didanosine. Although they do not investigate whether this association is present in their cohort, they highlight the fact that a number of other studies have observed such an association [3,4]. The authors rightly highlight the fact that use of didanosine and stavudine is less widespread in more recent years. Indeed, much of the stavudine and didanosine use in the EuroSIDA cohort is likely to be historical, rather than current. Awareness of the potential link between these antiretrovirals and pancreatitis may have led to less use of this combination as other nucleosides were developed, and to a reduction in the use in patients most susceptible to pancreatitis. Those susceptible to this complication may have already stopped the antiretroviral(s) prior to the study period, either because of the prior occurrence of pancreatitis, or because of other related issues.

In addition to the helpful suggestions made by Fessel and Hurley, we would also highlight the importance of applying consistent case definitions between studies so that results can be reliably compared. We have already begun further work, investigating the association between pancreatitis and triglycerides [5], and strongly agree that further research is needed in this subject area.

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Immune reconstitution syndrome to *Strongyloides stercoralis* infection

We thank McCarthy and Currie for their response [1] to our case report [2], which described a young Eritrean man with HIV and hepatitis B infections who presented with an inflammatory colitis, 6 weeks after starting antiretroviral therapy and had *Strongyloides* larvae detected in stool samples. After treatment with ivermectin, his symptoms, and the associated intestinal dilatation, resolved. His presentation was localized to the intestines and comprised loss of appetite, abdominal pain, vomiting and weight loss. There were no respiratory symptoms and no evidence of dissemination of *Strongyloides* larvae or bacterial sepsis.

We have seen and recognized the well established, strong association of corticosteroid treatment with *Strongyloides* hyperinfection syndrome [3–5] and agree that excluding *Strongyloides* infection is important when considering steroid treatment in individuals who have lived in an endemic area [1]. We also concur that immune suppression due to HIV infection itself does not appear to cause hyperinfection [6]. In this case, however, although this patient had received steroid treatment (for thrombocytopaenia) in the weeks preceding presentation, there was no evidence of dissemination of the parasite outside the gastrointestinal system, and therefore a local inflammatory response, coinciding with rapid immunological recovery due to antiretroviral treatment, fits much better with the clinical picture. We, therefore, proposed that this case comprises immune reconstitution syndrome and not disseminated infection secondary to corticosteroid therapy.

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