showed that the difference in CD4+ T cell count in the I.Co.N.A. Furthermore, they reported that they did not observe a difference in HCV RNA load by HCV genotype as well as by others [4–8]. Antonucci et al. [9] suggested several mechanisms that might explain these findings, including the possibility that HCV has a direct effect on CD4+ T cells [10], as we had proposed in our original study [2]. Although this possibility cannot be excluded, our data suggest that the relationship between HCV genotype and CD4+ T cell count is specific to those coinfected with HIV-1 and HCV, because no relationship between HCV genotype and either absolute or percentage CD4+ T cell measurements was identified in the HIV-1–uninfected participants in the HGDS cohort [2]. Further investigation is needed, to better define both the interactions between HIV-1 and HCV infection and how HCV RNA load and genotype influence the natural history of HIV-1 disease.

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Antimalarial Effects of HIV Protease Inhibitors: Common Compounds but Structurally Distinct Enzymes

To the Editor—The brief report by Skinner-Adams et al. [1] on the antimalarial effects of HIV protease (referred to as “protease” by Skinner-Adams et al.) inhibitors is of great interest and potential therapeutic importance in areas where malaria and HIV infection are coendemic. We were interested by the views expressed by these authors, as well as those expressed by Savarino et al. in their correspondence [2], regarding the aspartic proteinases of Plasmodium falciparum (plasmspsins) as likely targets for inhibition by these compounds. The work of both groups in this field is valuable, but greater caution should be exercised in the discussion of structural and sequence similarities between the 2 enzymes, because an appreciation of the molecular intricacies of the aspartic protease family will be essential for the proper interpretation of the antimalarial effects of HIV protease inhibitors.

The plasmspsins belong to aspartic protease family A1 [3], in which the 2 catalytic aspartic residues are contributed by different domains, generally formed from a single amino acid chain in a monomeric enzyme. A1 enzymes have long active site trenches overarched with a single flap, and are underlaid by a β sheet of 6 strands running approximately parallel to and beneath the active site trench. In contrast, HIV protease belongs to family A2 [3] and is a homodimeric enzyme that, consequently, has a symmetrical active site trench with 2 flaps. The active site trench of A2 enzymes is
much shorter than that of A1 enzymes, and
the dimer is held together on a base of 4
interdigitating strands, forming a β sheet
perpendicular to the active site trench. Thus,
although there may be some important
similarities in architecture between HIV
protease and plasmepsins, to discuss them
as being structurally similar is, in
reality, misleading. Consequently, we do
not share Savarino et al.’s surprise that
no plasmodial homologue of the HIV
protease was found, because there is no
true equivalent: the 9 P. falciparum as-
partic proteinases and the closely related
histoaspartic proteinase [4] are members
of the A1 protease family and are quite
distinct from the A2 retroviral-type di-
meric enzymes.

Furthermore, the sequence alignment
of HIV protease and plasmepsin II pre-
sented by Savarino et al. shows no more
similarity than would be expected for 2
diverse members of the aspartic proteinase
superfamily. The regions surrounding the
active site motifs of aspartic proteinases
are highly conserved, with a characteristic
hydrophobic–hydrophobic–aspartic acid–
threonine/serine–glycine sequence. In fact,
comparision of the leucine–aspartic acid–
threonine–glycine–serine sequence (plas-
mpxin II) with the isoleucine–aspartic acid–
threonine–glycine–alanine sequence
(HIV proteinase) illustrates the differences
between the enzymes as much as the sim-
ilarities, because the serine in plasmepsin
II is within hydrogen-bonding distance of
the catalytic aspartic acid, whereas the al-
anine in the equivalent position in HIV
protease cannot form such a bond.

The overall importance and interest of
these structural distinctions is that, assum-
ing the antimalarial activity of the anti-
retrovirals is mediated via the plasmepsins,
the inhibitors described are actually inter-
acting with active site trenches with very
different architectures—even if the model
produced by Skinner-Adams et al. indi-
cates that some HIV protease inhibitors
may adopt similar conformations on bind-
ing to both enzymes. These differences in
the characteristics of the active site trenches
are illustrated by distinct ligand-binding af-
nities—for example, the general aspartic
proteinase inhibitor 50-valeryl pepstatin in-
hibits plasmepsins with subnanomolar K
i values [5] but shows a K of 400 nmol/L
against HIV protease [6].

The inhibition of plasmepsins by the
HIV protease inhibitor ritonavir may
not be surprising, because interactions
between this drug and nonretroviral as-
partic proteinases have been known for
almost 10 years, with K values against
human cathepsins D and E of 20 and 8
mmol/L, respectively (see Kempf et al. [7],
whose work significantly predates that
cited by Savarino et al. in showing such
interactions). The plasmepsins are much
more closely related to human cathepsin
D than to HIV protease, so, when the
development of antimalarial therapy based
on HIV protease inhibitors is considered,
the potential interactions with human as-
partic proteinases may need to be mini-
mized. For ritonavir in particular this may
be of clinical relevance, because cathepsin
D is a lysosomal enzyme and, thus, is pres-
ent in most cell types and because cathepsin
E is found in epithelial cells lining the gut
(which are exposed to orally administered
drugs) and is also present in red blood
cells—the clinically significant site of ma-
larial infection. Saquinovir, by contrast,
achieves potent inhibition of HIV prote-
ase with negligible inhibition of the human
aspartic proteinases [8], demonstrating the
selectivity that can be achieved between
these types of enzymes. Ultimately, it may
be the structural differences governing the
interactions between inhibitors and the
plasmepsins (on the one hand) versus the
human aspartic proteinases (on the other)
that hold the key to the development of
existing drugs that target HIV protease as
antimalarial agents.

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