

another small-gatekeeper kinase, such as PKG, which is also important for invasion¹¹.

BKI-resistant and BKI-sensitive TgCDPK1 alleles will clearly be important tools for a more detailed dissection of TgCDPK1 function, as previously achieved for the “compound 1–PKG” pair. Apicomplexan and host PKGs have threonine (small) and glutamine (large) gatekeepers, respectively, and replacing the threonine with glutamine in the parasite’s enzyme abolishes its susceptibility to the inhibitor (this was elegantly exploited by the Donald and Baker groups to identify functions of the *T. gondii* and *Plasmodium* PKGs in the parasites’ life cycles)^{12–14}. Indeed, a number of apicomplexan enzymes have small gatekeeper residues, although the extreme case of a glycine is unique to TgCDPK1. If some of these enzymes are essential for parasite proliferation, the bump inhibitor is expected to have parasitocidal effects in the absence of any gatekeeper mutations, as now shown by Ojo and colleagues⁶. This might explain why it has not been possible so far to use the ASKA approach for functional studies of protein kinases in *Plasmodium* as had been proposed^{13,14}. Several recombinant *Plasmodium falciparum* kinases show the expected properties *in vitro*

(that is, susceptibility to bump inhibitors of small-gatekeeper mutants but not of the wild-type enzyme). It was hoped that an engineered glycine gatekeeper in an essential kinase would render the parasite much more susceptible to bump inhibitors, despite the presence of ‘threonine-gatekeeper kinases’ such as PKG. However, none of the several allele replacements in live parasites have resulted in a large increase in susceptibility to the bump inhibitor in cellular assays, although a modest decrease in IC₅₀ was observed in some instances (S. Eschenlauer, D. Dorin-Semlat, M.-P. Nivez, L. Reininger and C. Doerig, unpublished data). It should be possible to engineer parasite lines where the ‘natural’ small gatekeepers are substituted by large residues and to use such modified lines as a background for introducing ASKA mutations in other kinases whose wild types have large gatekeepers.

The two papers in this issue nicely illustrate that the phylogenetic distance between apicomplexans and their hosts is reflected by fundamental divergences in the properties of their respective protein kinases². In the case of TgCDPK1, both the atypical shape of the ATP binding pocket due to a glycine gatekeeper and the kinase’s unique activation mechanism offer

opportunities for the search of species-specific kinase inhibitors.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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HIV-1 autoreactive antibodies: are they good or bad for HIV-1 prevention?

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A new crystal structure of an anti-HIV-1 envelope antibody bound to an envelope-receptor complex shows the antibody binding both the HIV-1 envelope and the CD4 receptor, raising the question of what the role of antibody autoreactivity in host responses to HIV-1 may be.

The antibody response to HIV-1 seems to be peculiar in that, unlike influenza, neutralizing antibodies take a relatively long time (3–6 months) to develop against the transmitted/founder strain of HIV-1 (ref. 1); when they do first develop, HIV-1 envelope protein (Env) gp120 neutralizing antibodies that target the transmitted/founder virus are quite strain or type specific^{2,3}. Broadly neutralizing antibodies can develop in the course of HIV-1 infection,

but they do so only in a minority of subjects and only after several years of infection⁴. Passive protective trials with such rare broadly neutralizing antibodies have shown that these antibodies can prevent mucosal infection with simian immunodeficiency viruses expressing HIV-1 envelopes⁵. Thus, if broadly neutralizing antibodies could be routinely induced systemically and/or at mucosal sites, a practical HIV-1 vaccine would be in sight.

A recent HIV-1 vaccine trial in Thailand with a canarypox vector (called ALVAC) containing a clade AE_01 recombinant Env gp120 prime with a bivalent clade B and clade AE_01 gp120 boost showed 31% efficacy⁶. The lack of neutralization breadth induced by this type of vaccine has prompted the hypothesis that a type of non-neutralizing

antibody might provide some protection, perhaps by blocking the movement of virions or virus-infected cells across mucosal barriers and/or mediating antibody-dependent cellular cytotoxicity⁷. Thus, it remains to be determined what antibodies are ‘good’ and capable of being induced by experimental vaccine candidates.

Many of the rare broadly neutralizing antibodies that have been isolated have unusual traits, such as long heavy chain complementarity-determining regions (HCDR3s), high levels of somatic mutations and polyreactivity with a variety of host molecules⁸. Antibody polyreactivity is the ability to react with more than one antigen and is a normal component of the immunoglobulin repertoire. Approximately 60% of the preselection

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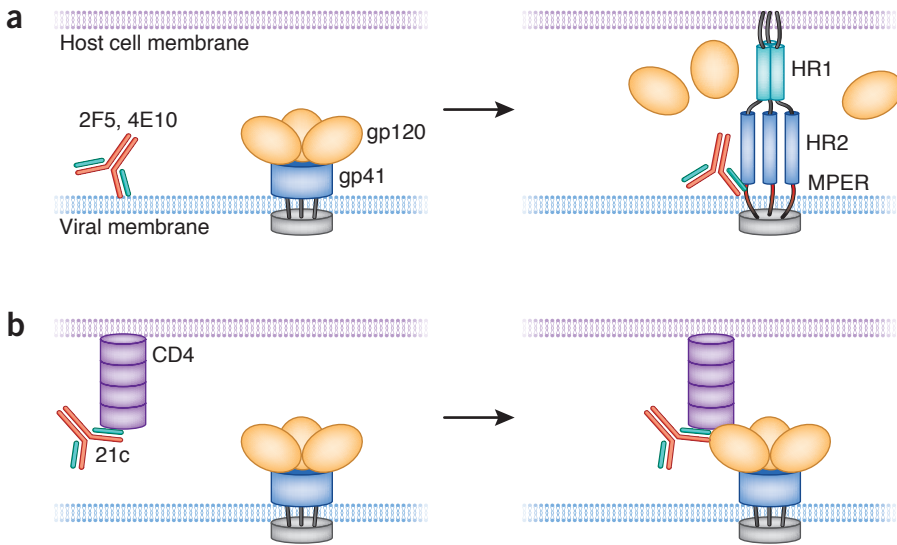


Figure 1 Possible modes of gp41 neutralizing and 21c monoclonal antibodies binding to the HIV-1 envelope. **(a)** 2F5 and 4E10 antibodies may initially interact with viral lipid membrane, which renders them readily available to bind their epitopes within the transiently exposed membrane-proximal external region (MPER) of the gp41 intermediate during the fusion process. Depicted in the gp41 intermediate state (right) are gp41 heptad repeat (HR) regions 1 and 2 and the MPER. **(b)** The antibody 21c may initially interact with CD4, which would efficiently position the antibody for a subsequent encounter with gp120. Alternatively, 21c may bind a pre-existing CD4–gp120 complex (right panel in **b**), in which case binding avidity would be a consequence of the autoreactivity of antibody 21c.

immunoglobulin repertoire is autoreactive⁹; most of these autoreactive B cells are removed during B-cell development, but as many as 20% of postselection B cells make autoantibodies, of which ~5% are polyreactive. However, polyreactivity has not been observed among the commonly made anti-HIV-1 gp120 CD4-inducible (CD4i)⁸ antibodies (due to their ability to recognize conformations of gp120 that are induced by binding CD4) that recognize the highly conserved CCR5 binding site¹⁰. Some of these antibodies derive from restricted VH families VH1-69 and VH1-24 (for example, 21c)¹¹. Although CD4i antibodies are reported to be induced by the type of vaccine used in the Thai trial¹⁰, they have not been considered to be strong candidates for induction by a preventive vaccine because whole CD4i antibodies do not neutralize HIV-1 well¹², whereas CD4i antibody Fab fragments do, implying partial occlusion of the CD4i antibody binding site¹³.

In this issue, Diskin, Marcovecchio and Bjorkman present the structure of a HIV-1 clade C envelope protein bound by both soluble CD4 and by a CD4i monoclonal antibody (21c) against the gp120 CCR5 coreceptor site¹⁴. Whereas most of the residues of the 21c HCDR3 make contacts to the CCR5 binding site on gp120, a small part of the HCDR3 and the 21c light chain L1 region binds CD4 in the CD4–gp120 complex. Thus, this is the first crystal structure to our knowledge of an

autoreactive antibody with its single Fab bound to more than one antigen at the same time. The antibody 21c has yet to be shown to react with host antigens other than CD4⁸, but its simultaneous binding to both a viral and host antigen is reminiscent of previously reported polyreactive HIV-1 antibodies (2F5 and 4E10), which bind membrane-proximal HIV-1 Env gp41 in complex with lipids, making contacts with both gp41 and membrane lipids^{15–18}.

This study raises a number of critical questions regarding the significance of the autoreactivity of anti-HIV-1 antibodies. First, is there functional significance behind the autoreactivity of the 21c and other HIV-1 antibodies? Both 2F5 and 4E10 have long hydrophobic HCDR3s, and mutation of those residues abrogates both lipid binding and HIV-1 neutralization while maintaining binding to gp41 (ref. 17). The binding of these mAbs to the gp41–lipid complex has been proposed as a sequential two-step process in which encountering the lipid membrane takes place first, presumably to help the antibody to dock with the transiently exposed gp41 intermediate neutralizing epitope during the virion–host cell fusion process^{17,18} (Fig. 1). The 21c antibody binds to some Envs in the absence of soluble CD4 (ref. 19), but it does not interact with the CAP210 clade C envelope unless soluble CD4 is present¹⁴. The 21c antibody reacts only weakly with soluble CD4 alone, raising the question of its biological

relevance *in vivo*. It remains to be determined whether the initial binding of 21c to CD4 is required for gp120 binding or the interaction of 21c with CD4 is an event that occurs when antibody 21c encounters CD4 bound to gp120 (Fig. 1). In the first case, an initial interaction with CD4 could position antibody 21c for a more efficient subsequent encounter with gp120. In the second case, simultaneous interaction with CD4 and gp120 could enhance the overall binding avidity of antibody 21c. A third possibility is that CD4 binding to gp120 induces new 21c reactive epitopes not previously present on either molecule alone.

Second, does antibody autoreactivity modulate the frequency of expression of antibodies like 21c? In human subjects infected with HIV-1, antibodies that react with the gp41 epitopes bound by antibodies 2F5 and 4E10 are rarely made. Because of their polyreactivity with lipids and other host proteins, it has been proposed that their expression is limited by central and peripheral tolerance mechanisms⁸. Indeed, in 2F5 and 4E10 VH knock-in mice, B-cell ontogeny analysis shows that a majority of these B cells are deleted during bone marrow B-cell development²⁰ (Verkoczy, L. and B.F.H., unpublished data). In contrast, CD4i antibodies that map to the CCR5 coreceptor binding region are extremely common, and most HIV-1–infected subjects develop non-neutralizing CD4i antibody specificities ~5–10 weeks after transmission¹. However, it is not known whether any of the many CD4i antibodies produced are autoreactive for CD4, nor is it known what the functional significance of such antibodies might be if they are made. As is pointed out by Diskin and colleagues, other CD4i antibodies such as 17b, 412d and X5 do not react with CD4, and we did not find polyreactivity in CD4i antibodies (including 21c) when screened against a series of host antigens⁸. Therefore, most types of CD4i antibodies are quite common and are unlikely to be regulated by tolerance mechanisms.

Third, are there any protective benefits derived from the induction of antibodies such as 21c by a preventive vaccine? The observation that CD4i antibodies do not neutralize transmitted/founder viruses, and that these antibodies are structurally constrained for access of whole antibody to the CCR5 binding site, have dampened enthusiasm for using the highly conserved CCR5 binding site as a vaccine target^{12,13}. On the other hand, recent observations indicate that strategies to target this highly conserved site might be found. Gray and colleagues²¹ and Li and colleagues²² have found, for clades C and B, select patients whose broadly neutralizing antibodies map to or overlap

the coreceptor binding site, respectively. CD4–gp120 complexes have been reported to induce neutralizing antibodies in monkeys²³, and induced CD4i antibody levels have been correlated with protection from simian-human immunodeficiency virus mucosal challenge in monkeys²⁴. If antibodies against CD4i epitopes can reach the surface of HIV-1 or HIV-1–infected cells, they could have salutary effects either by direct neutralization or by non-neutralizing mechanisms such as antibody-dependent cellular cytotoxicity²³.

Finally, the study of Diskin and colleagues is important because it raises the question of whether some of the commonly made antibodies induced by HIV-1 are autoreactive, as some of the rarely induced broadly neutralizing antibodies are. Autoreactive antibodies with low affinity for self antigens are commonly made in mice by B1 or marginal-zone B cells and are frequently derived by T cell–independent mechanisms. It will be

critical for those working on HIV-1 vaccine development to determine if the specificities of antibodies with low-affinity self reactivity are important to induce for a successful preventive vaccine or, alternatively, if these types of antibody responses should instead be avoided.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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