CCR5-tropic virus, some patients included in the studies had a viral tropism change from CCR5-tropic at screening to dual/mixed at baseline. A rapid outgrowth of preexisting archived X4 virus has also been demonstrated. Furthermore, resistance to maraviroc in patients failing with R5 virus has been demonstrated. In the future, the lack of maraviroc efficacy in R5 strains could be predicted by the presence at baseline of amino acids implicated in resistance; thus, V3 loop genotyping should be recommended before initiating CCR5 antagonist therapy.

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Antigenic mimicry of the HIV envelope by AIDS-associated pathogens

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Only one broadly neutralizing anti-HIV antibody, 2G12, recognises the envelope sugars of HIV. In the present study, we show that 2G12 also recognises *Candida albicans* and *Candida tropicalis* with high affinity (11 nmol/l) through a carbohydrate-dependent interaction (50% inhibitory concentration for D-fructose, 12 mmol/l). This is the first report of a neutralizing HIV antibody displaying cross-reactivity with another pathogen, revealing that the carbohydrate neutralization determinant of HIV, defined by 2G12, is more widespread amongst immunogenic, microbial surfaces than previously recognized

The carbohydrates that occlude most of the antigenic surface of HIV are attached to the viral envelope glycoproteins, gp120 and gp41, by the infected host cell [1]. They are therefore antigenically self and, almost without exception, immunologically silent. However, one anti-HIV-1 antibody, IgG1 2G12, has been shown to directly bind the HIV envelope glycans of HIV [1–8]. 2G12 can mediate antibody-dependent cell-mediated cytotoxicity of infected cells [8] and can protect against viral challenge by primary isolates S/HIV [9]. The search for an immunogen capable of eliciting 2G12-like antibodies remains a key goal in the design of a vaccine against HIV/AIDS [10].

The 2G12 F(ab')2 binds to oligomannose glycans of gp120 by recognition of a conserved Manα1-2Man motif [5–7,11]. Anti-Manα1-2Man antibodies are generally

abundant in mammalian sera [1,12] and can readily be elicited by immunization with synthetic [13] Manα1-2Man constructs. Similarly, biochemical [14] or genetic [15] inhibition of eukaryotic glycan biosynthesis provides diverse assemblies of 'self' oligomannose glycans that bind to 2G12. Again however, antibodies to these structures, although showing intriguing carbohydrate specificities [13,15], do not efficiently neutralize HIV-1. Despite repeated attempts, no immunization format has yet been shown to elicit neutralizing antibodies to gp120 oligomannose carbohydrates [16–18]. The only known carbohydrate neutralization motif on HIV, Manα1-2Man, remains antigenically silent in its natural context.

The extensive mutation [2] and the rare (apparently unique) specificity of 2G12 suggest that this antibody is unlikely to represent a 'natural' or germline antibody with an accidental cross-reactivity to self-glycans. Similarly, the 2G12 epitope is present on many HIV envelopes [19,20]; yet, 2G12-like antibodies are not normally elicited. Indeed, if such antibodies were generated in response to HIV infection, the 2G12 epitope would have been strongly selected against [21]. Thus, it is precisely because the 2G12 epitope is such a poor immunogen, that it is such an attractive target for vaccine design [1].

A carbohydrate vaccine for HIV-1 may therefore require a search for the Manα1-2Man motif in a more immunogenic format. One possibility is that 2G12 evolved to recognize a Manα1-2Man motif in an antigen other than $Man_9GlcNAc_2$ and, during the somatic hypermutation, lost tolerance to self-oligomannose structures. To investigate the plausibility of this hypothesis, we have determined the reactivity of 2G12 to alternative microbial antigens.

A large number of fungal mannans, including those associated with immunodeficiency [22], are based around a repeating Manα1-6Man backbone from which Manα1-2Man linkages branch. 2G12's affinity for mannoproteins from *Saccharomyces cerevisiae* $_{BY4742}$, *Candida glabrata* $_{2290E}$, *Candida tropicalis* $_{IGC2508}$, and *Candida albicans* $_{2005E}$ was determined by flow cytometry. The surface antigens of *S. cerevisiae* showed little or no affinity for 2G12. Of the three *Candida* species tested, *C. albicans* and *C. tropicalis* showed high affinities for 2G12, whereas *C. glabrata* lacked any detectable binding. No samples bound the control IgG b12. This demonstrates a degree of antigenic mimicry between these fungi and HIV gp120 that is specifically defined by 2G12.

The potent neutralizing activity of 2G12 against HIV is a function of its unusually high affinity for an antibody restricted to a carbohydrate epitope. The apparent affinity of 2G12 for *Candida* mannan is 11 nmol/l (Fig. 1b), which is comparable to the high affinity interaction of 2G12 for gp120 [3,7]. This indicates that the geometry, orientation, and clustering of mannose units on the surface of antigenic *Candida* is comparable to that found on HIV gp120. The reactivity of 2G12 for *Candida* is particularly surprising, given its highly selective requirements for linkages and spacing of carbohydrate residues on HIV gp120 [3,7,11].

To confirm the carbohydrate-dependent nature of the antigenic mimicry between *Candida* and HIV, the binding of 2G12 to the cell surface was determined in the presence of monosaccharide inhibitors of the carbohydrate-binding pocket of 2G12 [6]. The binding of 2G12 was inhibited by D-fructose, which is a known ligand for the 2G12 carbohydrate-binding site [6], but not by other monosaccharides such as D-glucose (Fig. 1b). The 50% inhibitory concentration for D-fructose (~12 mmol/l) corresponds to previously published observations on the interaction of 2G12 with gp120 [6], consistent with the inhibition of a similar molecular interaction (Fig. 1c). Taken together, these data demonstrate that 2G12 binds to *Candida* antigens in a highly specific manner, with an affinity similar to that of HIV, and through the carbohydrate-recognition domain on 2G12.

2G12 can recognize nonself arrangements of mannose units, but this does not mean that such arrangements will elicit 2G12-like antibodies [1,14,15,17,18]. Indeed, recent data suggest that, despite serving as a target for a broadly neutralizing antibody, the oligomannose glycans of HIV exhibit a direct immunosuppressive activity that limits antibody production [23]. Moreover, the unusual nature of the 2G12 Fab poses a challenge for templated vaccine design [1,24]. The data reported here might suggest clues to the origin of this so-far-unique antibody [1].

In addition to 2G12, other broadly neutralizing HIV antibodies display cross-reactivities to non-HIV antigens: 4E10 and, to a lesser extent, 2F5 [25,26] bind to 'self' lipids as well as the viral fusion protein, gp41 [27]. Although immune tolerance to 'self' antigens may not be a major barrier to the humoral response to HIV [26], polyspecificity nonetheless appears to be a shared feature of some broadly neutralizing antibodies, a property that could, in principle, be exploited in immunogen design [28].

Previous reports of antimannose antibodies, cross-reactive to gp120, have described polyclonal responses with low titres against laboratory-adapted HIV-1 gp120 [29,30]. Similarly the 'natural' human antimannose repertoire, which represents previous exposure to mannan-like antigens [22,31], does not generally neutralize HIV-1. These initial results discouraged the investigation of antimannose responses for vaccine research. However, the data reported here raise the possibility that immune responses to mannans might, under certain unknown circumstances, also include high-affinity monoclonal antibodies such as 2G12. The use of antimannan antibodies

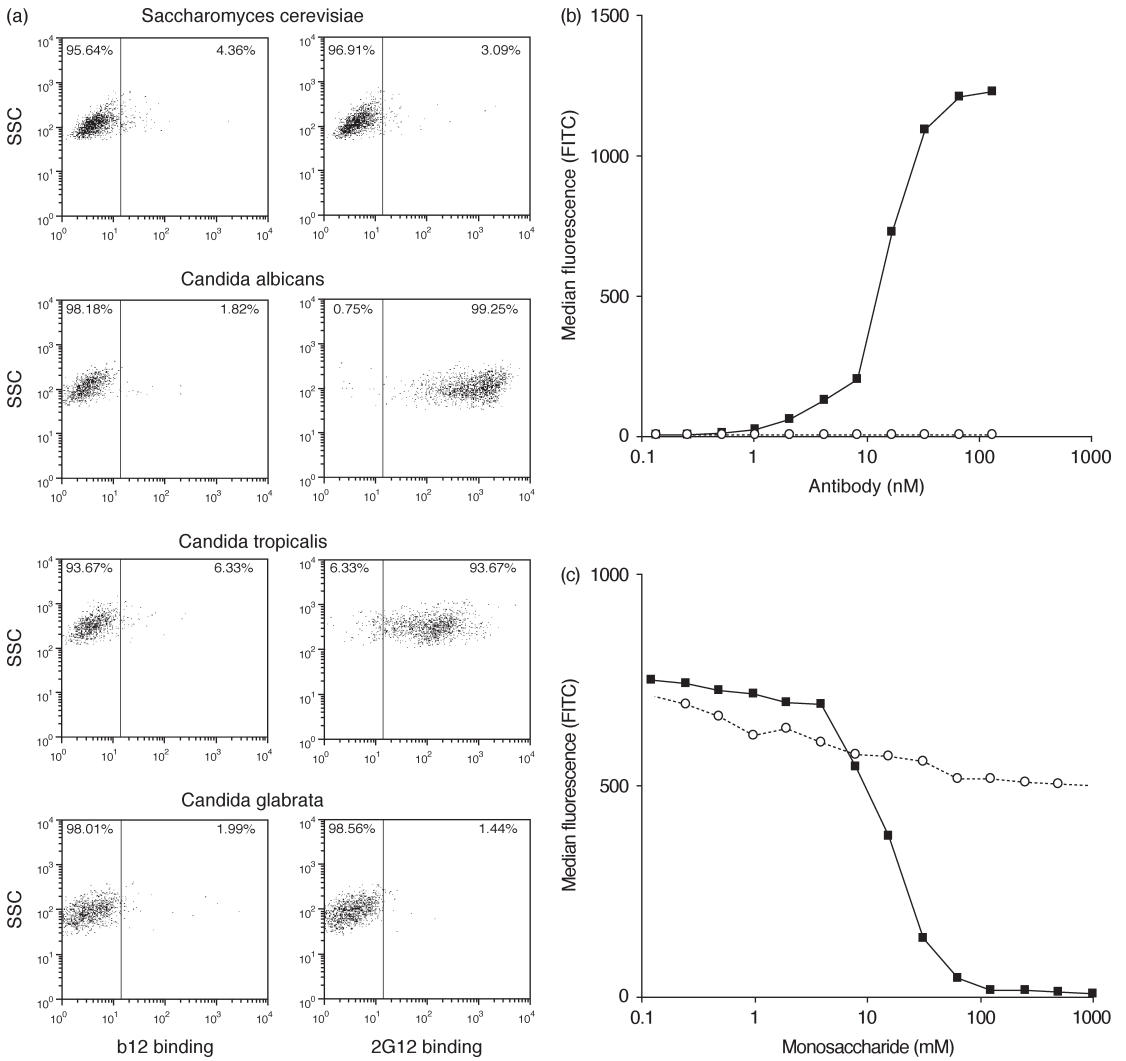

Fig. 1. The surface of some *Candida* species displays antigenic mimicry with HIV-1 gp120, as defined by immunoglobulin G 2G12. (a) The binding of immunoglobulin G (IgG) 2G12 and IgG b12 (both at 5 μg/ml) to the surfaces of *Saccharomyces cerevisiae* and various *Candida* species was determined by flow cytometry. Detection was by a FITC-labelled antihuman secondary antibody. (b) IgG 2G12 (—■—) and IgG b12 (—○—) were titrated at concentrations ranging from 0.01 to 20 μg/ml. 2G12 displayed an apparent affinity of 11 nmol/l for *Candida albicans* strain 2005E. (c) The interaction between 2G12 and *C. albicans* can be competed by high concentrations of a known competitor of the 2G12 carbohydrate recognition domain, D-fructose [6], (—■—) but not by D-glucose (—○—). All strains were grown at 30°C in YPD medium [1% (w/v) yeast extract; 2% (w/v) bactopeptone; 2% (w/v) dextrose] with 120 μg/ml kanamycin and shaken at 170 rpm. Cultures were harvested after 26 h. FITC, fluorescein isothiocyanate; SSC, side scatter.

as components of immune strategies against HIV-1 should perhaps be re-evaluated in the light of this result. Indeed, the evidence presented here indicates that the only known broadly neutralizing anticarbohydrate antibody against HIV might equally well be described as an anti-*Candida* antibody.

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