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Short communication

Analysis of the exposure of induced HIV glycoprotein epitopes in a potential HIV pseudovirion vaccine

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Abstract

Functionally conserved HIV-Env epitopes, which are induced during the process of Env-mediated membrane fusion, represent interesting immunogens, which may elicit broad neutralising antibody responses. In this report, we analyse a pseudovirion (PV)-based HIV vaccine preparation, potentially enriched in such induced Env-conformations. The vaccine has been prepared by mixing and incubating Env-PVs, with incorporated fusion-defective Env, with PVs, which have incorporated functional CD4 and CXCR4 proteins. Here, we demonstrate that three different monoclonal antibodies (CG10, 17b and 48d), recognising a region of gp120 overlapping with the coreceptor binding site, and a further antibody, 8F101, recognising a CD4-induced epitope outside of the coreceptor site, bind to Env molecules in the putative PV vaccine mixture but not at all, or less strongly, to native Env-PVs. In all cases, antibody binding required an interaction of the Env-PVs with CD4 whereas CXCR4 was dispensible. These results confirm that in the PV vaccine preparation, CD4-induced Env epitopes are accessible and that these, as well as other induced epitopes "downstream" from CD4 binding, may function as immunogens to elicit potentially cross-neutralising humoral immune responses.

Keywords: HIV vaccine; HIV pseudovirions; HIV-Env induced epitopes

1. Introduction

Although urgently required, a vaccine preventing HIV infection and/or AIDS is currently not available. Presently, most clinical trials evaluate vaccines, which are primarily aimed at eliciting cellular antiviral responses. However, since these responses do not protect from infection but only lower viral load and delay disease onset, it is likely that a successful HIV/AIDS vaccine formulation must contain components, which promote both humoral and cellular responses, including cross-clade virus neutralising antibodies. HIV Env is the target antigen against which neutralising antibodies are directed. Numerous strategies employing monomeric or trimeric Env proteins or native inactivated virions have failed to induce broadly neutralising responses (for review, see refs. [1,2]). This is largely due to the immense and rapidly developing sequence diversity of HIV in vivo, so that induced immune responses, effective against the HIV strain from which the Env vaccine has been derived, generally do not prevent infection by another HIV strain. Nevertheless, the existence of a small number of broadly neutralising monoclonal antibodies (Mabs), specifically b12 [3], 2G12 [4,5], 2F5 [4,6,7] and 4E10 [4,8], which have been generated from HIV/AIDS patients indicate that, in the course of the infection, the respective immunogen had been available. Thus, major efforts are presently being made to generate vaccine preparations enriched in these as yet unidentified, but presumably conserved, immunogens with the hope that these would

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lead to induction of high enough levels of broadly neutralising antibodies to positively impact protection and/or disease progression (for review, see refs. [2]).

It is possible that Env structures which exclusively arise during the dynamic interaction process of Env with the cellular HIV receptor complex may represent potent conformationally conserved immunogens for the induction of broadly cross-neutralising antibodies. These epitopes may be inaccessible in the native Env molecule and only be transiently exposed during the fusion process. Alternatively, and perhaps most importantly, epitopes, which are potential targets for cross-neutralisation, may be accessible but poorly immunogenic in the native Env molecule, yet display an increased immunogenicity in an intermediate Env conformation after interaction with the receptor complex. Several strategies, which aim to generate preparations enriched in "fusion intermediate" Env epitopes induced by interaction with the cellular receptor complex are being followed. Thus, cross-linked complexes between gp120/gp140 and soluble CD4 [9] or chimeric gp120-CD4 proteins [10] have been employed to target CD4-induced ($CD4_i$) epitopes on Env. In both reports, the sera of immunised animals exhibited cross-neutralising activity. However, whereas Fouts et al. present evidence supporting the view that the cross-neutralising activity was, at least in part, directed against Env [9], Varadarajan et al. demonstrate that the neutralising antibodies induced in their approach were exclusively against the CD4 component of the immunogen. Antibodies, with specificities for $CD4_i$ Env epitopes, were demonstrated to have been elicited but these were non-neutralising [10]. In a further approach, aimed at additionally targeting epitopes distinct from $CD4_i$ Env epitopes, tertiary complexes between gp120/gp140 and CD4 and coreceptor molecules [11] have been analysed in mice transgenic for human CD4 and CCR5. The induced antibodies were shown to be superior to those induced by Env proteins alone and to compete better with antibodies (X5, CG10), known to bind to induced Env epitopes.

In order for authentic "fusion intermediate" Env conformations to be induced, it may be necessary that the HIV-Env protein, rather than being soluble, be synthesised in mammalian cells in its native, membrane-bound form. Similarly, membrane-anchoring of the cellular receptor complex may also be required for its physiological interaction with the Env protein. For this reason, we aim to develop a vaccine strategy, which targets induced epitopes on membrane-bound Env. Non-infectious HIV pseudovirions (PV) have been chosen as a suitable platform for the presentation of membrane-bound Env to membrane-bound CD4 and coreceptor since these can be manipulated and defined in terms of protein content, amounts of incorporated Env, CD4, coreceptor, etc. Importantly, we [12,13] and others [14] have previously demonstrated that membrane fusion can occur between such populations of PVs which have incorporated wild-type HIV-Env (Env-PVs) and CD4/coreceptor (CD4/CXCR4-PVs or CD4/CCR5-PVs), respectively. This shows that Env molecules in the Env-PVs have undergone the full cascade of conformational changes required for fusion. In order for the induction of conformational change in Env to be terminated at an intermediate step prior to fusion, mutated Env, capable of binding to the receptor complex but unable to successfully complete fusion, is incorporated into the Env-PVs (now called Env^*-PVs). The Env presently used contains a single amino acid change within the fusion peptide of gp41 (gp41.2 [15,16]), a choice, which is empirical. Thus, the targeted Env immunogen, which is an induced intermediate Env conformation, is generated by incubating the respective Env^*-PVs and CD4/coreceptor-PVs together. This PV mixture represents the potential vaccine and will be analysed as to its ability to induce a humoral neutralising response in a huCD4/hu coreceptor transgenic rat model [17]. These animals are tolerant to the human CD4 and human coreceptor proteins in the potential vaccine and thus elicited neutralising antibodies should be directed against HIV-Env epitopes.

Since intermediate induced Env epitopes are the immunogens, which should be targeted in the planned PV vaccine strategy, it is of paramount importance to confirm that the Env^* protein in the PV vaccine has, in fact, undergone conformational change. Thus, in this communication, we have employed a panel of Mabs, specific for defined induced Env epitopes, to probe the PV vaccine and have confirmed that Env epitopes, which were absent in Env^*-PVs alone, have indeed been induced and are accessible in the PV vaccine.

2. Materials and methods

2.1. Constructs and proteins

PCHIV, referred to here as pcDNA-HIV, is an expression vector for the entire HIV genome except *nef* under control of the CMV promoter [18]. It contains the *Bss*HII–*Xho*I fragment (nucleotides 711–8887) from pNL4-3 [19] comprising the entire HIV coding sequence except for a portion of the *nef* gene, under control of the CMV promoter. The derivative, pcDNA-HIV-Env^{Fus-}, encodes for mutant Env with an exchange (V–E) at amino acid position 2 of the gp41 fusion peptide (from gp41.2 [15,16]) and additionally carries a specific C-terminal truncation (Tr752(N750K)), which, in the proviral context, leads to increased Env incorporation into virions [20]. This construct was cloned essentially by exchanging a *Nde*I-*Hin*dIII fragment (pNL 4-3 nucleotides 6399–8131) derived from fusion-defective pHenv41.2 and a *Hin*dIII-*Xho*I fragment (pNL 4-3 nucleotides 8131–8887) from pNL-Tr752(N750K) for the same fragments in pcDNA-HIV. The increased incorporation, established for proviral pNL-Tr752(N750K) also holds true in the context of pcDNA-HIV [21]. The derivative pcDNA-HIVΔEnv contains a frame-shift mutation at the beginning of the *env* gene, which abrogates Env expression [22]. pKCD4 is an expression vector for human CD4 [23] and pc.TEJ8-CXCR4ΔCT [24] encodes human CXCR4 with a deletion of 42 C-terminal

amino acids. This latter protein functions efficiently as HIV coreceptor. Recombinant HIV-1 IIIB gp120 produced in CHO cells was from ImmunoDiagnostics, Woburn, USA. sCD4-Hγ3 [25] was a kind gift from A. Traunecker, Basel Switzerland (used only in experiments with mouse Mabs) and soluble CD4 (sCD4) was purchased from Progenics Pharmaceuticals, New York.

2.2. Generation of HIV pseudovirions, preparation of potential immunogen

293T cells were transfected with pcDNA-HIV (to generate Env-PVs), with pcDNA-HIV-Env^{Fus-} (to generate Env*-PVs) or were cotransfected with pcDNA-HIVΔEnv and pKCD4 (to generate CD4-PVs) or with pcDNA-HIVΔEnv, pKCD4 and pc.TEJ8-CXCR4ΔCT (to generate CD4/CXCR4-PVs) using standard calcium phosphate transfection procedures. In order to be able to reduce the volume of the culture supernatant by ultrafiltration, the medium was replaced with DMEM, 0.5% FCS at 4–8 h p.t. and incubation continued for a further 40–60 h. The culture supernatants were then passed through a 0.45 μm filter, the volume reduced approximately 20-fold by ultrafiltration employing a Vivaflow 200 filter with a molecular weight cut-off of 100 KDa (Vivascience, Hannover, Germany), the PVs further concentrated by centrifugation at 200,000 × *g* for 3 h through a cushion of 20% sucrose in PBS and stored separately in aliquots at −70 °C. The amounts of the respective PVs were quantitated by HIV-1 p24 antigen capture ELISA (NCI-Frederick Cancer Research and Development Center, Frederick, USA) and by polyacrylamide gel electrophoresis followed by protein staining with Coomassie blue. Western blot analyses of PVs and of standard proteins were carried out using mouse HIV-CA Mab (H183) [26], rabbit anti-gp120 serum [27], mouse gp41 Mab (Chessie 8) [28], rabbit anti-CD4 and goat anti-CXCR4 (Santa Cruz Biotechnology). In order to generate the putative vaccine, Env*-PVs will be mixed and incubated with CD4/CXCR4-PVs essentially as described below but in the absence of Mabs.

2.3. Antibody binding to PVs

The following gp120 antibodies, which themselves are not broadly neutralising but which recognise defined induced Env epitopes, were employed as tools to probe Env conformation in PV preparations: mouse Mab CG10 [29] was provided by J. Gershoni, Tel Aviv, Israel, mouse Mab 8F101 [30] was provided by R. Pal, Advanced BioScience Lab, Inc., human Mab 17b [31] and human Mab 48d [32] were provided by J. Robinson, Tulane University, New Orleans.

The procedure to assess binding of the respective Mabs to PV preparations was essentially as described [33]. Briefly, Env*-PVs (equivalent 2.5 μg CA) either alone, mixed with sCD4 (10 μg), with CD4/CXCR4-PVs or, in some control experiments with CD4-PVs (both equivalent 5 μg CA) were incubated with either 200 μl culture supernatant from CG10 hybridoma cells (equiv. approximately 2 μg mouse IgG) or with 200 μl DMEM, 10% FCS containing 2 μg purified 17b, 48d or 8F101 IgG for 2 h at 37 °C in the presence of 8 μg/ml polybrene. In some experiments, prior to mixing with Env*-PVs, CD4/CXCR4-PVs were preincubated for 1 h at 37 °C with CD4 antibodies (200 μl of a 1:1 mixture of the culture supernatants of SIM-2 and SIM-4 hybridoma cells [34]). In further control experiments, 1 μg/ml AMD3100 (kindly supplied by D. Schols and E. deClerq, University of Leuven, Belgium), which blocks binding of gp120 to CXCR4, was added during incubation of the Env*-PVs with CD4-PVs. Subsequent to incubation of the PVs with the respective Mabs, the volume was increased to about 10 ml with DMEM, 10% FCS and layered on top of a 2 ml cushion of 20% sucrose in phosphate buffered saline (PBS) and centrifuged for 1 h at 29,000 rpm at 4 °C in a SW41 rotor (Beckman). The supernatant was carefully aspirated, the inside walls of the tube dried with tissue and the pellet dissolved in 120 μl 1% Empigen (Fluka) in PBS. The amounts of mouse or human IgGs associated with PVs in the dissolved pellet were quantitated by ELISA. Quantitative IgG ELISA was performed by coating wells of a multi-well plate with 50 μl 10 μg/ml goat anti-mouse IgG or goat anti-human IgG (Jackson company) overnight at 4 °C. The coated wells were washed five times with PBS/0.05% Tween/5 mM $MgCl_2$, then blocked with 1% BSA/5 mM $MgCl_2$/1% FCS in PBS for 1 h at 37 °C and subsequently washed five times. Samples, consisting of several dilutions of the dissolved pellets or standard amounts of mouse or human IgG, were applied to the wells and incubated for 1 h at 37 °C. After five further washes, 100 μl of biotinylated goat anti-mouse IgG or biotinylated anti-human IgG (Jackson/Dianova) at 0.5 μg/ml were added to each well and incubation continued for 1 h at 37 °C. After a further five washes, 100 μl of streptavidin-coupled alkaline phosphatase (2 μg/ml) were added, incubation continued for 30 min, the plates were then washed five times again and substrate consisting of 1 mg/ml *p*-nitrophenylphosphate (Calbiochem) in 10 mM diethanolamine/0.5 mM $MgCl_2$ pH 9.5 added. The OD at 405 nm was then determined.

3. Results and discussion

3.1. Preparation of Env*-PVs and CD4/CXCR4-PVs

Env*-PVs with incorporated fusion-defective Env, and CD4/CXCR4-PVs with incorporated functional CD4 and CXCR4 molecules were concentrated from the culture supernatant of transfected 293T cells. Gel electrophoresis and Coomassie blue staining showed that the HIV structural proteins CA, MA and NC were the major proteins in the PV preparations and that contamination with cellular proteins was low (not shown). Western blot analysis confirmed incorporation of gp120 and truncated gp41 (gp30) into Env*-PVs and incorporation of CD4 and CXCR4 into CD4/CXCR4-PVs. In the blot shown in Fig. 1, 100 ng CA, as determined

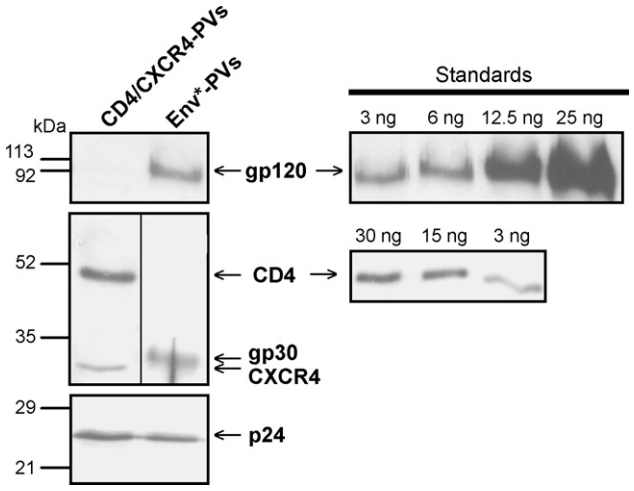

Fig. 1. Western blot analyses of PVs. Env*-PVs and CD4/CXCR4-PVs, each containing 100 ng CA, and standard amounts of gp120 and sCD4 have been analysed as indicated. The top part of the blot has been probed with rabbit anti-gp120, the left middle part with a mixture of mouse CD4 antibodies and goat anti-CXCR4, the right middle part with mouse gp41 Mab Chessie 8 and the bottom part with a mouse p24 Mab. The positions of molecular weight markers are shown on the left and of the detected proteins in the middle.

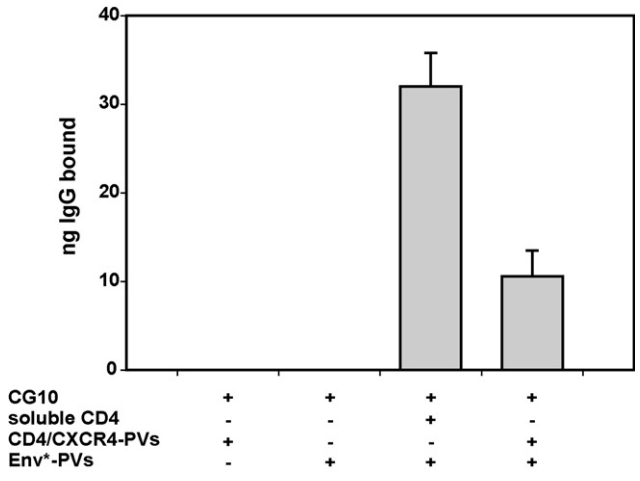

Fig. 2. Binding of mouse CG10 Mabs to PV preparations. Different mixtures (indicated below the graph) of Env*-PVs (equivalent 2.5 μg CA, containing approximately 125 ng gp120), CD4/CXCR4-PVs (equivalent 5 μg CA), sCD4-Hγ3 (10 μg) and CG10 Mab (2 μg IgG) were incubated for 2 h at 37 °C as described in Section 2. Subsequently, unbound antibodies were separated from the PVs by ultracentrifugation through a cushion of 20% sucrose. The total amounts of mouse IgG associated with the PV pellets were quantitated by ELISA and are given in ng IgG bound (total). The columns give the values and standard deviation from three experiments.

by CA-ELISA, of the respective PVs has been analysed. Densitometric analysis of the gp120 and CD4 band intensities, in comparison to protein standards, indicated that there was about 5 ng gp120 per 100 ng CA in Env*-PVs and 30 ng CD4 per 100 ng CA in CD4/CXCR4-PVs. The amount of incorporated CXCR4 was not quantitated. However, we have previously demonstrated in a comparable experimental set-up that coreceptor levels were sufficient to mediate virus particle fusion into cells expressing CXCR4-using HIV-Env [12,35].

3.2. Probing PV preparations with Mabs detecting induced Env epitopes

The putative PV vaccine is generated by mixing and incubating Env*-PVs with CD4/CXCR4-PVs. During this time, Env* proteins on the Env*-PVs are induced to undergo conformational changes which, due to the mutation in the fusion peptide of Env*-gp41, should terminate at an intermediate stage. This intermediate Env conformation is the targeted Env epitope in the putative PV vaccine. We have employed Mabs, specific for defined induced Env epitopes, to gain insight as to the extent and nature of the induced conformational changes in different PV preparations. After incubation of the respective Mab with defined amounts of PV preparations, unbound Mab IgG was removed from the PVs by centrifugation and the amounts of bound Mab IgG remaining in the PV pellet determined by ELISA.

3.2.1. Mouse Mab CG10

Mouse Mab CG10, which recognises a $CD4_i$ epitope on gp120 [29,31], was first used to probe CD4/CXCR4-PVs alone, Env*-PVs alone, Env*-PVs which were incubated with sCD4 and a mixture of Env*-PVs plus CD4/CXCR4-PVs (the latter represents the vaccine preparation). As shown in Fig. 2, neither CD4/CXCR4-PVs nor untreated Env*-PVs bound significant amounts of CG10 Mab which is consistent with a lack of presence and/or exposure of the respective epitope in native Env. However, Env*-PVs, which had been incubated with sCD4 and, importantly, Env*-PVs which had been incubated with CD4/CXCR4-PVs, clearly bound significant amounts of CG10 IgG (30 and 10 ng, respectively). On a molar basis, the amounts of bound CG10 IgG were less than the amounts of input gp120 in the Env*-PV aliquots analysed (containing 2.5 μg CA and thus 125 ng gp120). This may [36–38] or may not [39] be partially due to CD4-induced shedding of gp120. Alternatively, it may be due to some of the Env* molecules not having encountered CD4 on the CD4/CXCR4-PVs. Still further possibilities are that there is steric hindrance to binding of multiple IgGs to Env trimers or that induced Env epitopes, although present, may not be accessible for CG10 binding, a situation which has been reported to occur during cell–cell fusion [31,40]. In summary, the data obtained clearly show that in the PV vaccine, significant amounts of Env epitopes recognised by CG10 Mab have been induced and are accessible.

3.2.2. Human Mabs 17b and 48d

17b and 48d are two human Mabs, which have been reported to bind to a $CD4_i$ Env epitope, which overlaps with the coreceptor binding site [41]. Some 17b and 48d Mab binding to native Env has been shown to occur but this is significantly increased by addition of sCD4 [31,32]. In Fig. 3A, 17b Mab binding to PVs lacking Env, to Env*-PVs

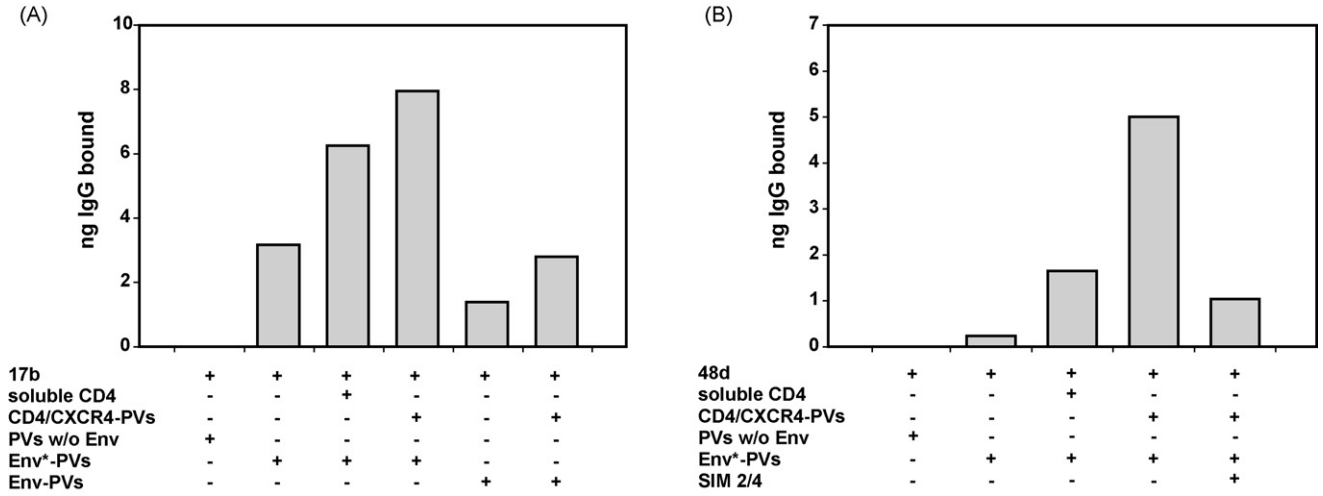

Fig. 3. Binding of human 17b and 48d Mabs to PV preparations. (A) Binding of 17b Mab. The compositions of the different mixtures analysed are shown below the graph. The amounts of PVs, of sCD4 and of 17b IgG and the procedure employed to analyse Mab binding are as described in Fig. 2. "PVs w/o Env" lack both Env and CD4/CXCR4 proteins, Env-PVs have incorporated Wt-Env (both equivalent 2.5 μg CA). The total amounts of human IgG associated with the PV pellets were quantitated by ELISA and are given in ng IgG bound (total). The columns show the result of one of two separate experiments, which gave similar results. (B) Binding of 48d Mab. The analyses were carried out as in (A). In the sample containing the mouse CD4 Mabs SIM-2 and SIM-4, these were preincubated with the CD4/CXCR4-PVs prior to mixing with the other components. The columns show the mean values obtained from two separate experiments.

alone, to Env*-PVs incubated with sCD4 or to Env*-PVs incubated with CD4/CXCR4-PVs, is shown. Whereas no binding of 17b Mabs to PVs lacking Env occurred, there was significant binding to Env*-PVs alone. Binding was increased approximately two-fold when the Env*-PVs were incubated with either sCD4 or CD4/CXCR4-PVs. This binding pattern was also observed employing Env-PVs, which had incorporated wild-type Env, although in this case, due to weaker Env incorporation into PVs (approximately three-fold lower, not shown), the amounts of bound 17b IgG were lower (Fig. 3A). This shows that, although truncations within the Env cytoplasmic C-terminus may affect the conformation of the Env ectodomain (e.g. [42]), the Tr752(N750K) truncation in Env* is not the reason for the relatively high level of 17b binding to Env*-PV in the absence of CD4. Fig. 3B shows similar analyses with 48d Mab. In this case, low binding of 48d Mab to Env*-PVs alone occurred and this was increased 7-fold and approximately 20-fold on incubation with sCD4 and CD4/CXCR4-PVs, respectively. In a further control experiment, CD4/CXCR4-PVs were preincubated with mouse anti-CD4 antibodies, which inhibit binding of CD4 to gp120, prior to incubation with the Env*-PVs and 48d Mab. As to be expected, this treatment significantly reduced 48d Mab binding to the PV vaccine preparation. These results with the human 17b and 48d Mabs confirm the results obtained using mouse CG10 Mabs, namely that $CD4_i$ Env epitopes are present and are accessible in the PV vaccine.

3.2.3. Mouse Mab 8F101

CXCR4 molecules are also present in the CD4/CXCR4-PV membrane and it is the aim of the proposed PV vaccine to additionally target Env epitopes, which are induced "downstream" of CD4 binding. We were thus interested in probing the different PV preparations with antibodies potentially specific for such epitopes. Mab 8F101 had been generated from a mouse immunised with cross-linked complexes of gp120 and sCD4 [30]. The 8F101 Mab recognises a $CD4_i$ epitope on Env outside the coreceptor binding site [40,43], i.e. a different epitope than is recognised by CG10, 17b and 48d. Although, 8F101 binds to soluble gp120-CD4 complexes, it has been reported that during cell–cell fusion, the 8F101 epitope is only presented in the context of the gp120-CD4-CXCR4 tricomplex [40]. As shown in Fig. 4, 8F101 Mab did not bind at all to PVs lacking Env or to Env*-PVs alone. Binding did, however, occur when Env*-PVs were incubated with either sCD4, CD4/CXCR4-PVs or CD4-PVs lacking CXCR4. Binding of 8F101 Mabs to Wt-Env-PVs treated with CD4-PVs also occurred (not shown) which excludes the possibility that the Tr752(N750K) truncation in Env* is the reason for the exposure of the 8F101 epitope in the absence of CXCR4. We also wanted to exclude the possibility that PVs had incorporated endogenous CXCR4 from the transfected 293T cells in amounts, which could be contributing to 8F101 binding. Although, CXCR4 protein expression is not detectable in these cells by Western blot or in indirect immunofluorescence analyses (not shown), low level CXCR4 expression must be occurring since 293T cells stably transfected with CD4 alone do support infection with T-cell tropic HIV, although inefficiently (data not shown). Thus, in a control experiment, AMD3100, which inhibits binding of gp120 to CXCR4, was added during incubation of the Env*-PVs with CD4-PVs but this treatment did not reduce the level of bound 8F101 IgG (not shown). In summary, these results mean that, in the context of PVs, exposure of the 8F101 epi-

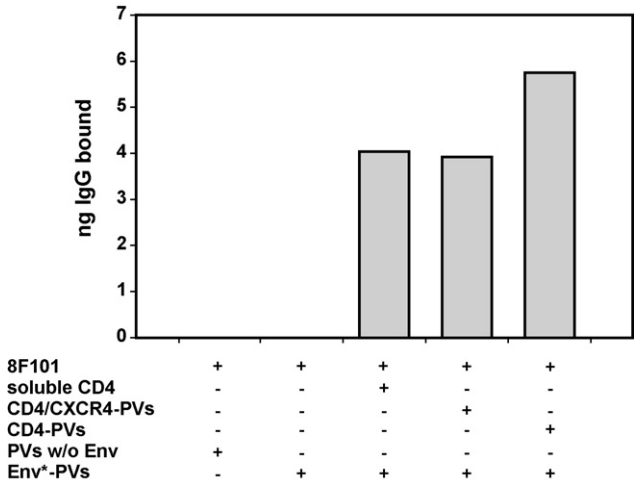

Fig. 4. Binding of mouse 8F101 Mabs to PV preparations. The compositions of the different mixtures analysed are shown below the graph. The amounts of PVs, of sCD4-Hγ3 and of 8F101 IgG and the procedure employed to analyse Mab binding are as described in Fig. 2. The columns show the mean values obtained from two separate experiments.

tope is induced by CD4 binding alone. The reason for this difference to the situation during cell–cell fusion when 8F101 epitope exposure requires CXCR4 [40], is unclear. It could be that during cell–cell fusion, the binding site for 8F101 Mab is actually also generated by CD4 binding alone but, in contrast to the situation in PVs, is not accessible prior to CXCR4 binding.

The purpose of this study had been to confirm that in preparations of Env*-PVs, mixed and incubated with CD4/CXCR4-PVs (the "PV vaccine"), new Env epitopes, absent in the Env*-PVs alone, were present. The binding studies, employing the antibodies described above, confirm that this is the case and that different types of CD4 induced epitopes (recognised by CG10, 17b, 48d and by 8F101, respectively) are accessible. None of the Mabs employed are broadly neutralising and it is unlikely that exposure of CD4-induced epitopes per se will be sufficient to create an Env immunogen for the induction of broadly neutralising antibodies. However, in the strategy employed here, CXCR4 is additionally incorporated into the CD4/CXCR4-PVs (Fig. 1) and in previous studies employing Wt-Env, we have shown that its amount is sufficient to allow membrane fusion. Thus, in the PV vaccine, binding of functional CXCR4 will induce further conformational changes in mutant Env* which, however, should terminate at an intermediate stage prior to membrane fusion. Unfortunately, antibodies specific for conformations "downstream" of $CD4_i$ epitopes on gp120, but still prior to formation of the gp41 six-helix bundle, are not available. Thus, it is empirical which Env mutation may lead to the most relevant induced conformation. Immunisation experiments in suitable animal models (initially in rats transgenic for human CD4 and coreceptor [17,44]) will reveal if the PV vaccines described here, are able to elicit a relevant humoral response.

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