Deficient class-switched IgG antibody response to the HIV CD4 binding site and neutralizing IgG synthesis induced by covalent immunization

MOPE0045

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ABSTRACT

Background: Adaptive immune response upon gp120 and HIV infection is mostly directed to the hypermutable and immunodominant variable domains of gp120. In contrast, the gp120 CD4 binding site (CD4BS), an epitope essential for HIV life cycle and part of the gp120 superantigen determinant is poorly immunogenic.

Methods: HIV infected subjects sera were from the San Francisco Health Men study. BALB/cJ mice were immunized with gp120 and electrophilic gp120 (E-gp120). Antibody binding activity was tested by ELISA. Antibodies were purified by affinity purification using immobilized anti-μ and Protein G columns.

Results: Affinity purified IgM and IgG from HIV infected subjects were tested for binding to gp120 and the electrophilic gp120 416-433 region (E-416-433), a mimetic of the CD4BS. IgMs but not IgGs bind to E-416-433. The IgM binding to E-416-433 was inhibited by E-416-433 and gp120 but not by irrelevant control protein, indicating that the IgM binding is specific. Similarly, IgMs but not IgGs from three gp120 immunizations bind specifically E-416-433. These data indicates a deficiency of IgM→IgG class-switching of the antibody response to the CD4BS region in humans with HIV infection and mice immunized with gp120. In contrast, IgM and IgG response from mice immunized with the covalently reactive E-gp120 displayed robust and specific binding to the CD4BS. CD4BS binding by IgM from gp120 and E-gp120 immunized mice were equal and IgM response to the CD4BS, increased progressively upon multiple gp120 injections.

Conclusions: This is the first direct evidence of B cell downregulation by gp120. HIV infection and gp120 immunization is characterized by a conventional adaptive maturation of the CD4BS during HIV infection and gp120 immunization in IgM antibody class and a deficient IgM to IgG class-switching of CD4BS specific B cell. Covalent immunization with E-gp120 allows the IgM to IgG class-switching to the poorly immunogenic and superantigenic CD4BS.

INTRODUCTION

- Eradication of human immunodeficiency virus (HIV) will require an effective vaccine. Previous test vaccines have induced antibodies (Abs) to the HIV coat protein gp120 regions that are highly mutable or are non-essential for the viral life-cycle.
- The mostly-conserved gp120 determinant necessary for binding the primary host cell receptor CD4, the CD4 binding site (CD4BS), is poorly immunogenic. Consequently, Abs that neutralize genetically divergent HIV strains are rarely produced after HIV infection or after immunization with previously tested experimental vaccines.
- The CD4BS is a discontinuous determinant. The CD4BS core (CD4BS^{core}) composed of residues 421-433 is located on the gp120 surface and plays an essential role in CD4 recognition (1). As this is a conformationally flexible region, its accurate mimicry by synthetic peptides has been difficult. We developed a novel probe to analyze the production of anti-CD4BS^{core} Abs. The probe, E-416-433, is composed of the consensus residues 416-433 with an electrophilic phosphonate attached to Lys421 and Lys432 side chains. Despite the absence of certain CD4BS residues located in the gp120 outer domain, E-416-433 can bind CD4 autonomously, consistent with the importance of the CD4BS^{core} in the recognition of CD4 (refs 2 and 3; see also Fig 1).
- Here we describe deficient production of IgG antibodies to the CD4BS^{core} in the blood of HIV infected patients and mice immunized with recombinant gp120, the active ingredient of AIDSVAX tested previously as an HIV vaccine.
- The electrophilic analog of gp120 (E-gp120), in contrast, induced robust production of IgG antibodies to the CD4BS^{core}. E-gp120 was previously found to induce the production of broadly neutralizing monoclonal Abs (2).

METHODS

- **Electrophilic probes** (**Fig 1).** Refs 2 and 4 describe preparation and characteristics of electrophilic probes of full length gp120 (subtype B MN strain; E-gp120), synthetic peptide analogs of the CD4BS^{core} (E-416-433**a**), and the irrelevant vasoactive intestinal peptide (VIP) containing phosphonate diesters at Lys side chains and biotin at the N terminus. BSA and KLH conjugated to E-416-433 (E-416-433**b** and E-416-433**c**) and E-VIP were prepared as in 3. cbz-Z is an electrophilic hapten containing the same phosphonate group present in E-416-433 but devoid of the 416-433 peptide (5).
- **Immunization.** BALB/c mice were hyperimmunized with gp120 or E-gp120 intranasally or intraperitoneally. Subjects 0.5-5 years post infection were obtained from the San Francisco Health Men's study cohort. Samples from non-infected subjects were from the Gulf Coast Blood Bank, Houston, Texas.
- **ELISA.** Serum IgM and IgG bound to immobilized antigens in ELISA plates were detected using peroxidase conjugated anti-μ and γ chain IgG, respectively. Competitive ELISA was performed by monitoring bound Abs in absence or presence of the following soluble polypeptides: gp120, sCD4, E-416-433 along with control ovalbumin and E-VIP peptide.
- **Ab purification.** IgG and IgM was purified by affinity chromatography using immobilized Protein G and immobilized anti-μ chain Abs, respectively. Protein concentration was measured with BCA.
- **HIV neutralization:** This was determined by p24 measurement following infection of peripheral blood mononuclear cells by clinical HIV isolates (6).

RESULTS

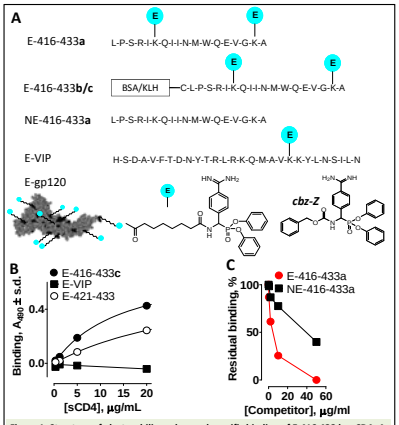

Figure 1: Structure of electrophilic probes and specific binding of E-416-433 by sCD4. *A.* E denotes the phosphonate group located at Lys residue side chains. E-416-433a contains an N terminal biotin group, E-416-433**b** is the BSA-conjugate and E-416-433**c** is the KLH- conjugate (2,3). The hapten cbz-Z blocks covalent antibody binding by reacting irreversibly with antibody nucleophiles but does not impair non-covalent antibody binding, permitting measurement of non-covalent binding free of any contribution due to covalent binding or catalysis at the Ab nucleophilic sites. ***B,*** Specific binding of sCD4 to immobilized E-416-433**c** and the shorter E-421-433. E-416-433**c** and KLH-E-421-433, 0.3pmol/well. sCD4 binding determined using rabbit anti-CD4 Ab in the presence of 100 μM cbz-Z. E-VIP, an irrelevant electrophilic peptide (4). ***C,*** Competitive inhibition of sCD4 binding to immobilized E-416-433**c** by soluble E-416-433**a** and NE-416-433**a** epitope mimetics. NE-416-433**a**, non-electrophilic biotinylated peptide without phosphonate groups. sCD4 binding was determined as in panel B.

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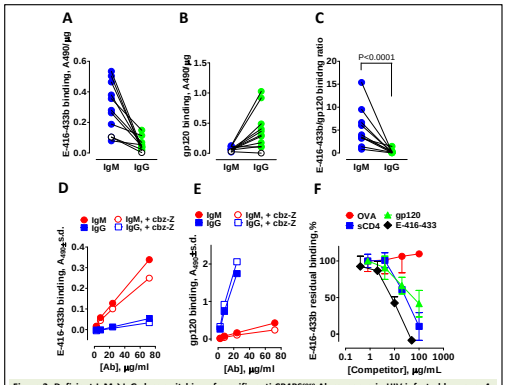

Figure 2: Deficient IgM→IgG class switching of specific anti-CB4BS^{core} Ab response in HIV-infected humans. *A*, Binding of E-416-433**b** by IgM or IgG purified from the sera of HIV infected patients 0.5-5 years post-seroconversion determined by ELISA (n=10). The data are expressed as A490/μg Ab. Abs, 30 μg/ml. Each closed symbol represents the indicated Ab fraction from one patient. Open circles, pooled Igs from 11 noninfected subjects. ***B,*** Binding of full length gp120 by the same human IgM and IgG samples as in panel A. The data are expressed as A490/μg Ab. IgG 10 μg/ml; IgM 30 μg/ml. ***C,*** E-416-433**b**/gp120 binding ratios for human IgM and IgG computed from Panels A and B. Mann-Whitney U-test, two tailed. Note lower values for IgG compared to IgM, indicating selective impairment of anti-CD4BS^{core} Ab class switching. ***D,*** Dose-dependent binding of donor 2089 IgM and IgG to E-416-433**b** in presence or absence of cbz-Z (100 μM), suggesting that most of the binding is attributable to non-covalent forces . ***E,*** Dose-dependent binding of donor 2089 IgM and IgG to gp120 in presence or absence of cbz-Z (100 μM). ***F,*** Example curves showing residual E-416-433**b** binding by IgM from subject 1932 in the presence of increasing concentration of sCD4, gp120, E-416-433**a** and control ovalbumin. Control E-VIP did not reduce IgM binding to E-416-433 (data not shown).

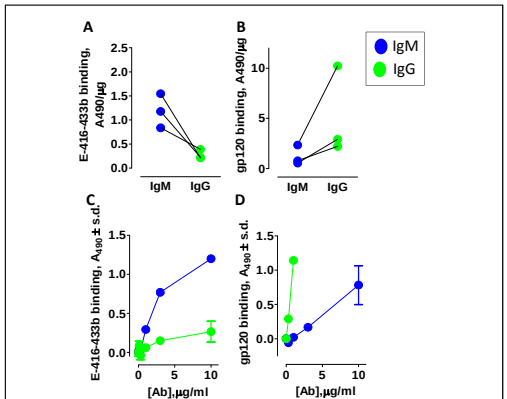

Figure 3: Deficient IgM→IgG class switching of specific anti-CD4BS^{core} Ab response in gp120-immunized mice. Binding assays were performed in presence of the electrophilic phosphonate hapten (cbz-Z, 100 μM). ***A,*** Binding of E-416-433**b** by IgM or IgG from the sera of gp120 immunized mice. Shown are the results of 3 immunizations using pooled sera from 4-10 mice/immunization. ***B,*** Binding of full length gp120 by IgM or IgG from the same sera shown in Panel A. ***C,*** Example of concentration-dependent E-416-433**b** binding by serum IgM and IgG induced by immunization with gp120. ***D,*** Example of concentration-dependent gp120 binding by serum IgM and IgG induced by immunization with gp120.

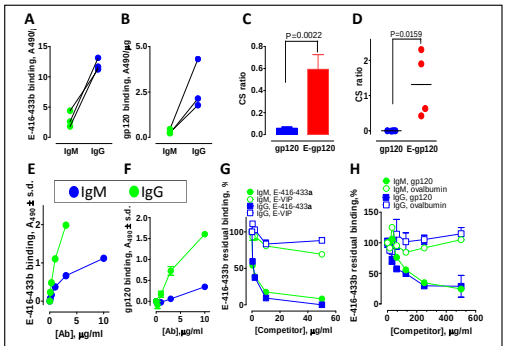

Figure 4: Efficient IgM→IgG class switching of the anti-CD4BS^{core} Ab response induced by immunization of mice with E-gp120. ELISAs were conducted in presence of 100 μM cbz-Z. ***A,*** Binding of E-416-433**b** by IgM or IgG from the sera of E-gp120 immunized mice. Shown are the results of 3 immunizations using pooled sera from groups 2-10 mice/immunization. ***B,*** Binding of full-length gp120 by IgM or IgG from the same sera shown in Panel A. ***C,*** Relative IgM→IgG class-switch (CS) ratio for the gp120 and E-gp120 induced Ab responses. Plotted are the CS ratios for the anti-CD4BS^{core} Ab response/anti-gp120 response, defined as [(A490 E-416-433**b** binding per μg IgG/A490 E-416-433**b** binding per μg IgM) ÷ (A490 gp120 binding per μg IgG/A490 gp120 binding per μg IgM A490)]. A value of 1.0 on the y-axis indicates that class switch efficiency for the anti-CD4BS^{core} response is equivalent to the average class switch efficiency for the immunodominant epitopes expressed by full-length gp120. Values are means ± SEM for pooled sera from 3 independent immunizations each as shown in Fig 3 and the present Figure. ***D,*** Reproducibly induction of efficient anti-CD4BS^{core} Ab class switching by E-gp120 in individual mice. Plotted are the CS ratios for the anti-CD4BS^{core} Ab response/anti-gp120 response from one each of the immunizations in Fig 3 and Fig (n=4 mice for each immunogen). ***E,*** Example of concentration-dependent E-416-433**b** binding by serum IgM and IgG induced by immunization with E-gp120. ***F,*** Example of concentration-dependent gp120 binding by serum IgM and IgG induced by immunization with E-gp120. ***G,*** Example curves showing residual E-416-433**b** binding by IgM and IgG from pooled serum of E-gp120 immunized mice in the presence of increasing concentration of E-416-433**a** or control E-VIP. ***H,*** Residual gp120 binding by IgM and IgG from pooled serum of the E-gp120 immunized mice in the presence of increasing concentration of gp120 and control ovalbumin.

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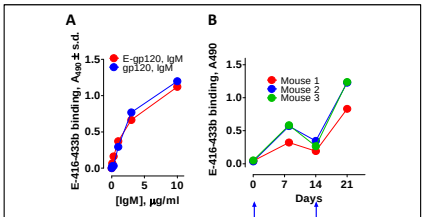

Figure 5: Equivalent anti-CD4BS^{core} IgM responses to gp120 and E-gp120. ***A***, Concentration-dependent E-416-433**b** binding by pooled IgM from mice immunized with gp120 and E-gp120 (n=10 for each immunogen). ***B***, Amplified E-416-433**b** binding IgM response upon a booster gp120 immunization. Arrows indicate gp120 administrations. Serum dilution 1:500.

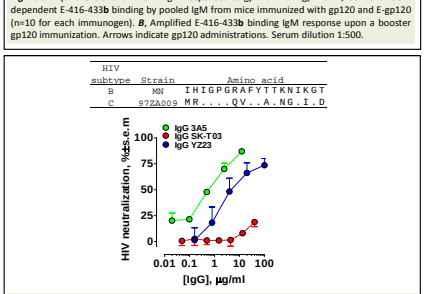

Figure 6: Heterologous HIV neutralization by monoclonal IgGs raised by E-gp120 immunization. ***Top,*** Aligned sequences of the immunodominant 307-325 region epitope (HXB2 numbering) of the E-gp120 immunogen (subtype B strain MN) and the heterologous CCR5-dependent subtype C HIV isolate 97ZA009 tested for neutralization. •, identities; #, unidentified residues. ***Bottom,*** Representative strain 97ZA009 neutralization data at varying concentrations of anti-E-gp120 monoclonal IgGs YZ23 and 3A5 directed to the CD4BS^{core} and clone IgG SK-T03 directed to a non-neutralizing gp120 epitope. Values are means of 4 cultures ± S.D.

RESULTS

- **Fig 1** shows the structure of E-416-433, the probe used to identify anti-CD4BS^{core} Abs, along with the control irrelevant E-VIP probe and the E-gp120 immunogen. Noncovalent Ab binding occurs at the peptide epitopes. Nucleophiles located in the Ab binding sites bind the electrophilic phosphonate group covalently (7). E-416-433 binds soluble CD4 (sCD4) specifically at levels 120x-superior to previously described synthetic peptide mimetics of the CD4BS^{core} (8, 9).
- **Fig 2** shows limited binding of E-416-433 by IgG Abs from HIV infected patients compared to IgM Abs. In contrast, IgG Abs from the same patients displayed superior binding to full-length gp120 compared to IgM Abs, consistent with a robust and mature Ab response to the non-CD4BS^{core} immunodominant epitopes of the protein. Specificity of the CD4BS^{core} Abs was evident by inhibition observed using competitor soluble gp120, sCD4 and E-416-433 but not ovalbumin or E-VIP. The data suggest impaired IgM → IgG class switching of Ab directed to the CD4BS^{core}.
- **Fig 3** shows that mice immunized with gp120 also displayed impaired IgM→IgG class switching of the anti-CD4BS^{core} Abs. The impairment was evident both in the intranasal and intraperitoneal immunization protocol.
- **Fig 4** shows that the problem of impaired IgM→IgG class switching is solved when the immunization is conducted using E-gp120 instead of gp120. E-gp120 administered either intranasally or intraperitoneally induced robust anti-CD4BS^{core} IgG responses. Competition experiments demonstrated that the E-gp120 induced Abs were specific for the CD4BS^{core}.
- **Fig 5** shows that the anti-CD4BS^{core} Ab response to gp120 and E-gp120 in the IgM compartment was comparable. No suppression of the gp120-induced IgM response was evident upon administration of a booster immunogen injection. Therefore, there is no evidence of impaired anti-CD4BS^{core} IgM in response to gp120 immunization.
- Ref 2 reported that E-gp120 induces monoclonal Abs to the CD4BS^{core} with HIV neutralizing activity. **Fig 6** shows example anti-E-gp120 monoclonal Abs with ability to neutralize a heterologous HIV-1 strain.

CONCLUSIONS

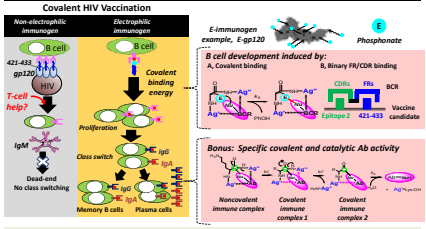

Figure 7: Implications of covalent B cell stimulation for HIV vaccination. Traditional non-electrophilic immunogens induce a transient Ab response to the CD4BS^{core} epitope limited mostly to the IgM compartment because of deficient class-switching (*left panel*). The electrophilic phosphonate of the prototype E-vaccine binds BCR nucleophiles (*Nu*) *via* the highly energetic covalent reaction, bypassing constraints on B cell differentiation (middle panel). This generates memory B cells and plasma cells producing neutralizing Abs. An optional epitope 2 that generates a positive signal by binding the CDRs can be incorporated in the E-vaccine to counteract negative B cell signaling due to 416-433 epitope binding at the FRs (upper pink panel). Electrophile-driven clonal selection of the B cells results in adaptive strengthening of Ab nucleophilic reactivity, improving the innate catalytic activity of Abs (lower pink panel). Specificity is derived from noncovalent epitope-paratope binding. Covalent immune complex 1, resonant stable complex prior to expulsion of C-terminal antigen fragment. Covalent immune complex 2, acyl-Ab complex. Ag'Lys-OH, N-terminal antigen fragment; NH2-Ag'', C-terminal antigen fragment.

- **IgM Abs are the first Abs produced following stimulation of B cells with microbial antigens. As the response matures, class switching to IgG antibodies occurs together with improvement of antigen recognition due to adaptive sequence diversification. We observed impaired IgM→IgG class switching of the anti-CD4BS^{core} Abs in HIV infected humans and mice immunized with gp120. This explains the rare production of mature neutralizing Abs to this region.**
- **Efficient anti-microbial defense is dependent on production of class-switched IgG/IgA Abs. Evidently, HIV has developed a means to limit production of mature neutralizing Abs to the CD4BS^{core}. The CD4BS^{core} has displayed superantigenic character in reports from several groups, a property thought to downregulate the adaptive immune response (10-12). A strong IgM response and weak class-switched IgG response has also been reported for other B cell superantigens (13).**
- **E-gp120, an immunogen capable of covalent binding to the B cell receptor (Ab expressed on B cells), induced robust IgM→IgG class switching of the anti-CD4BS^{core}. As proposed previously, the large amount of binding energy released due to the covalent binding may channel B cells into a productive response that is physiologically forbidden (Fig 7).**
- **The covalent vaccination strategy may be suitable for developing an effective vaccine that stimulates neutralizing antibodies to the CD4BS (see Abstract TUAA0101).**