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Antibodies to the superantigenic site of HIV-1 gp120: Hydrolytic and binding activities of the light chain subunit

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Abstract

Antibodies (Abs) to the superantigenic determinant of HIV gp120 ($gp120_{SAg}$) are potential protective agents against HIV infection. We report that the light chain subunits of Abs cloned from lupus patients using phage library methods bind and hydrolyze $gp120_{SAg}$ independent of the heavy chain. Unlike frequent $gp120_{SAg}$ recognition by intact Abs attributable to V_H domain structural elements, the isolated light chains expressed this activity rarely. Four light chains capable of $gp120_{SAg}$ recognition were identified by fractionating phage displayed light chains using peptide probes containing gp120 residues 421–433, a $gp120_{SAg}$ component. Three light chains expressed non-covalent $gp120_{SAg}$ binding and one expressed $gp120_{SAg}$ hydrolyzing activity. The hydrolytic light chain was isolated by covalent phage fractionation using an electrophilic analog of residues 421–433. This light chain hydrolyzed a reporter $gp120_{SAg}$ substrate and full-length gp120. Other peptide substrates and proteins were hydrolyzed at lower rates or not at all. Consistent with the expected nucleophilic mechanism of hydrolysis, the light chain reacted selectively and covalently with the electrophilic $gp120_{SAg}$ peptide analog. The hydrolytic reaction entailed a fast initial step followed by a slower rate limiting step, suggesting rapid substrate acylation and slow deacylation. All four $gp120_{SAg}$-recognizing light chains contained sequence diversifications relative to their germline gene counterparts. These observations indicate that in rare instances, the light chain subunit can bind and hydrolyze $gp120_{SAg}$ without the participation of the heavy chain. The pairing of such light chains with heavy chains capable of $gp120_{SAg}$ recognition represents a potential mechanism for generating protective Abs with enhanced HIV binding strength and anti-viral proteolytic activity.
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Keywords: B cell superantigen; HIV gp120; Catalytic antibody light chain; Phage library; Lupus

1. Introduction

Certain Abs are thought to bind the HIV-1 coat protein gp120 with no requirement for V domain somatic sequence diversification, leading to classification of gp120 as a B cell superantigen (Berberian et al., 1993; Townsley-Fuchs et al., 1997). Synthetic gp120 peptides composed of residues 241–250, 331–360 and 421–440 (strain MN numbering) inhibit Ab binding to the gp120 superantigenic epitope (hereafter designated $gp120_{SAg}$), suggesting that $gp120_{SAg}$ is a conformational determinant (Goodglick et al., 1995). The region corresponding to residues 421–433 is of particular interest because of its conserved sequence in diverse HIV isolates[1] and its role in HIV binding to CD4, the primary receptor mediating viral entry into

Abbreviations: Ab, antibody; AMC, 7-amino-4-methylcoumarin; BCR, B cell receptor; BSA, bovine serum albumin; CDR, complementary determining region; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; EP, electrophile; FR, framework region; $gp120_{SAg}$, superantigenic determinant of gp120; HERV, human endogenous retrovirus; HIV-1, human immunodeficiency virus type 1; PBS, phosphate buffered saline; t_R, retention time; SDS, sodium dodecylsulfate; sEGFR, soluble epidermal growth factor receptor; TFA, trifluoroacetic acid; V domain, variable domain

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[1] Percent conservation of residues gp120 421–433 in 550 HIV strains belonging to various clades available in the Los Alamos Database is: A (54) 93%; B (155), 95%; C (111) 97%; D (20) 96%; F (10), 93%; G (11) 90%; CRF (189) 94% (alphabetical letters are clade designations and numbers in parentheses are numbers of strains; CRF, circulating recombinant forms). For each strain, the number of identities with the consensus residues in the 421–433 epitope (K-Q-I-I/V-N-M-W-Q-E/R/G-V-G-K/Q/R-A) were counted. % conservation was calculated as 100 × (number of identities)/total number of residues in the peptide epitope.

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host cells. The importance of this region is suggested by reports that: (a) CD4 binding by gp120 is compromised following site-directed mutagenesis at residue 421, 427 and 430 (Olshevsky et al., 1990); (b) contacts between soluble CD4 and certain residues within the 421–433 region are evident by X-ray crystallography (Kwong et al., 1998); (c) trypsin-catalyzed cleavage of gp120 at the 432–433 peptide bond results in loss of CD4 binding activity (Pollard et al., 1991).

In view of the functional role of the $gp120_{SAg}$ in HIV entry, anti-$gp120_{SAg}$ Abs can be hypothesized to help protect against infection. In support of this hypothesis, high level $gp120_{SAg}$ binding by IgG from a subpopulation of HIV-seronegative individuals is correlated negatively with the incidence of subsequent HIV infection (Townsley-Fuchs et al., 1996). However, there is no evidence that exposure to HIV induces the production of protective Abs with improved $gp120_{SAg}$ recognition capability. To the contrary, superantigens are thought to induce B cell apoptosis (Goodyear and Silverman, 2003; Goodyear and Silverman, 2004; Goodyear et al., 2004), and production of V_H3+ family immunoglobulins that bind superantigens is reduced in HIV infected subjects (Berberian et al., 1991; Juompan et al., 1998; Scamurra et al., 2000). Interestingly, patients with the autoimmune disease systemic lupus erythematosus display increased levels of Abs that bind the 421–436 peptide component of $gp120_{SAg}$ (Bermas et al., 1994; Zhou et al., 2002). Clinical case reports have commented on the low frequency of coexistent lupus and HIV infection (Palacios et al., 2002; Daikh and Holyst, 2001), and we have described a single chain Fv (scFv, Ab V_L and V_H domains linked by a short peptide) isolated from the expressed lupus repertoire that binds the 421–436 region and neutralizes HIV infection of peripheral blood mononuclear cells in tissue culture (Karle et al., 2004).

Abs to $gp120_{SAg}$ have another distinctive property—a subpopulation of the Abs can hydrolyze gp120 (Paul et al., 2004). As fragmentation of polypeptides can result in permanent loss of their biological function, this property confers increased biological potency to the Abs compared to their reversibly binding counterparts (Berisha et al., 2002). Abs generally catalyze peptide bond hydrolysis by a mechanism similar to conventional serine proteases, *i.e.*, nucleophilic attack on the carbonyl group, formation of a transient covalent intermediate and hydrolysis of the intermediate (Paul et al., 2006). The activated nucleophile responsible for initiation of the catalytic reaction has been localized to the light chain subunit of certain Abs by mutagenesis (Gao et al., 1995) and crystallography methods (Ramsland et al., 2006), and additional reports indicate the ability of light chains to catalyze chemical reactions independent of the heavy chain subunit (e.g., Mei et al., 1991; Matsuura et al., 1994; Paul et al., 1995; Tyutyulkova et al., 1996; Mitsuda et al., 2004; Hifumi et al., 2005). Electrophilic phosphonate haptens originally developed to bind irreversibly to the catalytic residues of conventional serine proteases (Powers et al., 2002) have been applied to isolate proteolytic Ab fragments displayed on phage surfaces (Paul et al., 2001). The haptens do not discriminate between antigen-specific and promiscuous proteolytic Abs, but inclusion of a peptide epitope neighboring the electrophile permits noncovalent epitope–paratope interactions, imparting specificity to the covalent reaction of the electrophile with Ab nucleophiles (Taguchi et al., 2002; Planque et al., 2003; Nishiyama et al., 2004).

The binding of $gp120_{SAg}$ has been thought to result from interactions with Ab V_H domains, with little or no contribution from the V_L domain (Karray et al., 1998; Neshat et al., 2000). We report here certain rare light chains isolated from the phage displayed repertoire of lupus patients that bind $gp120_{SAg}$ without dependence on the heavy chain subunit. One light chain isolated based on its reaction with an electrophilic analog of the superantigenic epitope peptide 421–433 displayed the ability to selectively cleave a $gp120_{SAg}$ reporter substrate and full-length gp120. The pairing of such light chains with known $gp120_{SAg}$ binding heavy chains is a promising route to protective Abs with enhanced HIV binding strength and anti-viral proteolytic activity.

2. Materials and methods

2.1. EPs, substrates and synthetic peptides

The syntheses and Ab binding characteristics of the electrophilic compounds have been reported: **EP1** (Nishiyama et al., 2002), **EP2a** and **EP2b** (Taguchi et al., 2002; Planque et al., 2003). The $gp120_{SAg}$ peptide substrate **3** was prepared by standard solid phase peptide synthesis using Fmoc-Lys-(AL resin)-AMC (Fmoc, 9-fluorenylmethoxycarbonyl; Anaspec, CA), followed by deprotection with TFA, purification by reversed-phase HPLC, and lyophilization. Its identity and purity were confirmed by HPLC [t_R 25.32 min, >99.9% (YMC ODS-AM); 0.05% TFA in water:0.05% TFA in MeCN 80:20–20:80 in 60 min, 1.0 ml/min; 220 nm absorbance], electrospray-ionization mass spectrometry [observed *m*/*z*, 1673.8 and 837.2; calculated $(M+H)^+$ and $(M+2H)^{2+}$ for $C_{78}H_{117}N_{19}O_{20}S$, 1674.0 and 837.5], and amino acid analysis of the acid hydrolysate [Asx 1.0 (1), Glx 2.9 (3), Gly 0.9 (1), Val 1.1 (1), Met 0.9 (1), Ile 1.6 (2), Lys 2.1 (2); peptide content 69%]. Preparation of the $gp120_{SAg}$ peptide **4** corresponding to residues 421–436 is described in a previous paper (Karle et al., 2003). An irrelevant control peptide corresponding to residues 351–364 of the human epidermal growth factor receptor was obtained by similar means. Following peptide-AMC substrates were from Peptides International (Louisville, KY): Lys-AMC; Phe-AMC; Arg-AMC; Ala-AMC; Suc-Ala-Glu-AMC (Suc, succinyl; Ala-Glu-AMC); Suc-Ala-Ala-Ala-AMC (Ala-Ala-Ala-AMC); Suc-Ile-Ile-Trp-AMC (Ile-Ile-Trp-AMC); Suc-Ala-Ala-Pro-Phe-AMC (Ala-Ala-Pro-Phe-AMC); Boc-Glu-Lys-Lys-AMC (Boc, *tert*-butoxycarbonyl; Glu-Lys-Lys-AMC); Boc-Val-Leu-Lys-AMC (Val-Leu-Lys-AMC); Boc-Glu(O-Bzl)-Ala-Arg-AMC (Bzl, benzyl; Glu-Ala-Arg-AMC); Boc-Ile-Glu-Gly-Arg (Ile-Glu-Gly-Arg-AMC); Pro-Phe-Arg-AMC; Z-Gly-Gly-Arg (Z, benzyloxycarbonyl; Gly-Gly-Arg-AMC); Z-Gly-Gly-Leu (Gly-Gly-Leu-AMC). Purified gp120 (strain MN expressed in the baculovirus system; Immunodiagnostics, Woburn, MA), sEGFR and BSA were labeled with 6-biotinamidohexanoyl at Lys residues (1–2 mol biotin/mol protein) as described previously (Planque et al., 2003; Paul et al., 2003).

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