EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Neutralizing Antibodies Against a Specific Human Immunodeficiency Virus gp41 Epitope are Associated With Long-term Non-progressor Status

Olivier Lucar [a], Bin Su [b,c], Valérie Potard [d], Assia Samri [a], Brigitte Autran [a], Christiane Moog [b], Patrice Debré [a], Vincent Vieillard [a,*]

[a] Sorbonne Universités, UPMC Univ Paris 06, INSERM U1135, CNRS ERL8255, Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), Paris, France
[b] U1109 INSERM, FMTS, Université de Strasbourg, Strasbourg, France
[c] Center for Infectious Diseases, Beijing You'an Hospital, Capital Medical University, Beijing 100069, China
[d] Sorbonne Universités, UPMC Univ Paris 06, UMR-S 1136, Paris, France

ARTICLE INFO

Article history:
Received 13 January 2017
Received in revised form 7 July 2017
Accepted 7 July 2017
Available online xxxx

Keywords:
HIV-1
Long-term non-progressors
Neutralizing antibody

ABSTRACT

Antibodies (Abs) play a central role in human immunodeficiency virus (HIV) protection due to their multiple functional inhibitory activities. W614A-3S Abs recognize a specific form of a highly conserved motif of the gp41 envelope protein and can elicit viral neutralization to protect $CD4^+$ T cells. Here, we describe in detail the neutralizing profile of W614A-3S Abs in untreated long-term non-progressor (LTNP) HIV-infected patients. W614A-3S Abs were detected in 23.5% (16/68) of untreated LTNP patients compared with <5% (5/104) of HIV-1 progressor patients. The W614A-3S Abs had efficient neutralizing activity that inhibited transmitted founder primary viruses and exhibited Fc-mediated inhibitory functions at low concentrations in primary monocyte-derived macrophages. The neutralizing capacity of W614A-3S Abs was inversely correlated with viral load ($r = -0.9013$; $p < 0.0001$), viral DNA ($r = -0.7696$; $p = 0.0005$) and was associated the preservation of high $CD4^+$ T-cell counts and T-cell responses. This study demonstrates that W614A-3S neutralizing Abs may confer a crucial advantage to LTNP patients. These results provide insights for both pathophysiological research and the development of vaccine strategies.

1. Introduction

Neutralizing antibodies (NAbs) are a good correlate of protection in infectious diseases such as yellow fever, smallpox, and measles (Amanna et al., 2008). The potential protective role of Nabs during the course of human immunodeficiency virus (HIV) infection remains a highly debated issue. Many HIV-infected individuals naturally develop NAbs that target several sites on the gp41 and gp120 HIV-1 envelope proteins after several years of infection, but only 10–25% develop potent and broadly reactive Nabs (Mikell et al., 2011; Hraber et al., 2014; West et al., 2014). These findings suggest that the human immune system can achieve NAb responses, but whether these Abs are naturally protective during HIV infection remains unclear.

Studies of NAbs in general have provided an enormous impetus to HIV vaccine research and to immunology as a whole (Sadanand et al., 2016). Monoclonal Abs (mAbs) with the remarkable ability to neutralize most circulating strains of HIV-1 were recently isolated from HIV-infected individuals. Examples include 3BNC117 and PGT121 mAbs, which both transiently block infection and suppress viremia in simian/HIV (SHIV)-infected macaques, demonstrating that the passive administration of potent NAbs protects macaques for a short period of time (Barouch et al., 2013). In HIV-1-infected humans, a single infusion of 3BNC117 mAb, which specifically targets the CD4-binding site on gp120, decreased viremia for up to 28 days (Caskey et al., 2015).

Although these data indicate the potential effectiveness of passive immunotherapy by NAbs, the successful development of preventive HIV-1 vaccines requires a thorough understanding of how Nabs are induced during HIV-1 infection. The unusual maturation features of NAbs make them extremely difficult to induce and indicate that one of the most important challenges for vaccines development is to characterize well-defined targets that specifically stimulate neutralizing activity (Sadanand et al., 2016). The gp41 subunit is far more conserved than gp120, and the fusion machinery is common to all strains. We previously described a highly specific motif localized in a gp41 HIV-1 region, called 3S (Vieillard et al., 2005; Vieillard et al., 2016), localized between the N-terminal heptad repeat (HR) 1 and the HR2, that appears to be exposed to the surface in the trimeric pre-fusion structure of the HIV-1 envelope (Fig. S1), in line with recent resolve structures of the HIV-1 envelope trimer (Pancera et al., 2014; Lee et al., 2016). In an *in vivo* macaque model, immunization with a candidate vaccine based on the 3S motif induced non-neutralizing Abs, which limited $CD4^+$ T-cell

* Corresponding author.
E-mail address: vincent.vieillard@upmc.fr (V. Vieillard).

http://dx.doi.org/10.1016/j.ebiom.2017.07.007

depletion, immune activation, and inflammation, thus achieving immune protection and restoring immune homeostasis (Vieillard et al., 2008, 2012).

An alanine-scanning assay within the 3S motif of the viral gp41 protein showed that a tryptophan residue at position 614 (W614) is crucial for the virus entry (Petitdemange et al., 2013). The main reason could be that this region plays a key role in the formation of the six-helix bundled gp41 ectodomain core structure that imposes several kinetic and steric constraints responsible for the high degree of motif preservation, as previously reported (Gallo et al., 2003). Altogether, these data could explain the absence of detectable "3S" escape variants, and the remarkable conservation of the 3S motif within the gp41 (Vieillard et al., 2005; Potard et al., 2013). Next, we generated in mice a class of Abs against the 3S motif, called W614A-3S Ab that elicits neutralizing activity, against a panel of tier 1 and tier 2 viruses from clades A, B, C and E (Petitdemange et al., 2013). More recently, these data were confirmed in rabbit and macaque models (unpublished data are from Vieillard et al.). Our data are in accordance with Bradley et al. (2016) showing that amino-acid changes in gp41 membrane proximal region (MPER) induce viral neutralization sensitivity. Interestingly, we also determined that approximately 5% of HIV-1-infected progressor patients naturally produce neutralizing W614A-3S Abs (Petitdemange et al., 2013).

In this study, we analyzed the neutralizing activity of W614A-3S Abs isolated from long-term non-progressor (LTNP) patients from the French ALT ("Asymptomatic Long-Term") cohort ("Agence Nationale de Recherche sur le Sida" ANRS CO15). These HIV-1-infected individuals account for <0.4% of the total HIV population (Grabar et al., 1999). They maintain high $CD4^+$ T-cell counts and remain therapy naïve. Here, we report an unusually high frequency of LTNP patients with W614A-3S Nabs. These Abs displayed neutralizing activities over a five-year follow-up period, which are correlated with low viral load, low viral reservoir, and high $CD4^+$ T-cell responses. These data support the hypothesis that these specific Nabs play an important role *in vivo* in maintaining LTNP status.

2. Materials and Methods

2.1. Study Subjects

This study enrolled 68 LTNP HIV-1^+ patients from the French ALT cohort (ANRS CO15). Their characteristics are summarized in Table 1. As previously reported, ALT cohort members met the following inclusion criteria: HIV seropositivity for at least 8 years and CD4 cell counts > 600/mm^3 for the past 5 years, whatever the viral load, but without symptoms or antiretroviral therapy (Grabar et al., 1999). LTNP patients were subdivided into three groups according to their capacity to produce neutralizing W614A-3S Abs, non-neutralizing WT-$3S^+$ Ab only, or neither subtype (Neg). The CO15 ALT cohort is funded and sponsored by ANRS and was approved by the ethics review committee of Ile de France – VI. All patients provided written informed consent, and all methods were performed in accordance with relevant guidelines and regulations indicated by the Declaration of Helsinki.

2.2. CD4 Count and Viral Production

The course of the immune and virological status of LTNP patients from the ALT cohort has been described elsewhere (Candotti et al., 1999; Magierowska et al., 1999; Martinez et al., 2005). All tests were conducted in a single laboratory. CD4 cell counts were performed on fresh blood by flow cytometry (Coulter) and were determined in accordance with a standard internal control (mean ± SD reference value, 858 ± 260 cells/mm^3). HIV-1 RNA viral load was quantified in fresh plasma samples using an ultrasensitive HIV-1 Amplicor-Monitor assay (Roche-Diagnostic Systems; limit of detection, 20 copies/ml). The level of HIV-1 viral DNA was determined in frozen peripheral blood mononuclear cells (PBMCs) using a modified Amplicor Monitor assay (Roche Laboratories) with an internal HIV-1 proviral DNA standard. Results were expressed as copies of HIV-1 viral DNA/10^6 PBMCs with a limit of detection of 1 copy/10^6 PBMCs.

Sequencing analysis of the patient viral sequences at the 614 position of the 3S motif is shown in Table S1. This analysis came from the

Table 1
Characteristics of study ALT patients.

Characteristics	Neg	WT-3S	W614-3S	P*
Number	24	28	16	
Median age in years	36.0	36.6	38.0	0.55
Male:Female (n)	20:4	24:4	8:8	**0.02**
Median viral load (quartiles)	9.3×10^3 (2.1×10^3–1.0×10^5)	2.3×10^5 (4.5×10^3–1.0×10^5)	1.3×10^2 (3.3×10^1–4.1×10^2)	<**0.0001**
Median viral DNA (quartiles)	403 (120–1449)	430 (114–1011)	24 (8–27)	<**0.0001**
Median CD4 count (quartiles)	605 (467–769)	717 (609–799)	697 (590–833)	0.09
Median % CD4 (quartiles)	33.0 (27.5–37.0)	30.0 (26.0–36.0)	39.5 (35.0–42.5)	**0.01**
In $CD4^+$ T cells (%, median, quartiles)				
HLA-DR^+	8.5 (4.0–9.0)	8.5 (6.0–14.0)	6.0 (3.0–11.0)	0.12
$CD38^+$	76.5 (70.0–86.0)	76.0 (58.0–80.5)	69.5 (50.0–75.0)	0.07
$CD45Ra^+$	59.0 (49.0–65.0)	53.0 (43.5–91.0)	51.0 (41.0–68.0)	0.24
$CD45Ro^+$	47.0 (35.0–58.5)	50.0 (35.5–76.0)	51.0 (39.0–66.0)	0.81
Median CD8 count (quartiles)	818 (685–964)	1121 (974–1541)	717 (531–1168)	**0.004**
Median % CD8 (quartiles)	42.5 (38.0–49.5)	48.0 (41.5–60.0)	39.0 (36.0–44.0)	**0.004**
In $CD8^+$ T cells (%, median, quartiles)				
HLA-DR^+	27.0 (19.0–35.0)	33.0 (28.0–45.0)	22.0 (16.0–30.0)	**0.007**
$CD38^+$	56.5 (45.0–69.0)	51.0 (38.0–66.0)	43.5 (37.0–50.0)	**0.03**
$CD45Ra^+$	65.0 (54.0–74.5)	54.0 (46.0–70.0)	64.0 (55.0–74.0)	0.24
$CD45Ro^+$	28.5 (20.2–41.2)	35.0 (24.2–45.7)	30.0 (21.0–38.0)	0.52
$CD57^+$	38.0 (34.0–52.0)	34.0 (26.5–83.0)	34.0 (20.0–43.0)	0.49

Significant values ($P < 0.05$) expressed in *bold*.
* Statistical analysis was performed using chi-square test for categorical variables and Kruskal-Wallis test for continuous variables, with Dunn post-test.

Please cite this article as: Lucar, O., et al., Neutralizing Antibodies Against a Specific Human Immunodeficiency Virus gp41 Epitope are Associated With Long-term Non-progressor ..., EBioMedicine (2017), http://dx.doi.org/10.1016/j.ebiom.2017.07.007