Journal of Virological Methods 158 (2009) 70–76

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

A model for testing the immunogenicity of simian immunodeficiency virus and simian–human immunodeficiency virus vaccine candidates in mice

Jun Xu [a], Matthew Kelly [a], Warren Denning [b], Zdenek Hel [a,b,*]

[a] Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA
[b] Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, USA

Article history:
Received 22 October 2008
Received in revised form 16 January 2009
Accepted 21 January 2009
Available online 30 January 2009

Keywords:
HIV-1
SIV
SHIV
Vaccine
DNA vaccine
Recombinant viral vaccine
$CD4^+$ T cell
$CD8^+$ T cell
T cell epitope

A B S T R A C T

HIV-1 Gag protein represents a promising target of cellular immunity-based vaccines due to its immunogenicity and high conservation among diverse viral subtypes. Development of novel and effective Gag-targeted vaccine candidates inducing $CD8^+$ and $CD4^+$ T cell responses requires large scale pre-clinical testing in a small animal model. In this report, the MHC class I and II-restricted epitopes in the simian immunodeficiency virus (SIV) Gag protein recognized in C57Bl/6 and Balb/c mice were determined and characterized. In addition, using the newly defined epitopes, the relationship is described between the amount of plasmid DNA, volume of inoculate, and the extent of ensuing immune responses following intramuscular DNA immunization.

1. Introduction

Cytotoxic T lymphocytes (CTLs) play a pivotal role in the control of HIV-1 infection in humans and simian immunodeficiency virus (SIV) infection in macaques (Jin et al., 1999; Matano et al., 1998; Goulder and Watkins, 2008). Although the vaccines based on the induction of cellular immune responses do not protect against the acquisition of the virus, the presence and rapid expansion of virus-specific CTLs at the time of infection results in a state of immune control restricting viral proliferation and decelerating progression to disease (Hel et al., 2002, 2006a,b). Despite the recent setbacks in the HIV-1 vaccine field, results obtained using highly pathogenic SIV infection in macaques suggest that vaccine-induced cellular immunity can exert considerable control over replication of an immunodeficiency virus in a complete absence of Env-specific neutralizing antibodies (Watkins et al., 2008). Elicitation and maintenance of both CTL and humoral responses is dependent on help from $CD4^+$ helper T cells (Bevan, 2004). $CD4^+$ helper T cells support a prompt response to acute infection as well as long-term control of chronic infection. We and others have demonstrated a key role of vaccine-induced $CD4^+$ T cells in conferring protection against immunodeficiency viruses (Hel et al., 2000, 2002, 2006b; Tryniszewska et al., 2002; Letvin et al., 2006; Villinger et al., 2002). However, migration of activated virus-specific $CD4^+$ T cells to the site of infection may fuel viral proliferation by providing highly susceptible target cell population (Staprans et al., 2004). Therefore, the net effect of vaccine-induced $CD4^+$ T cells on HIV-1/SIV infection depends on their tissue distribution and functional characteristics.

A number of approaches aiming to enhance the frequency of $CD4^+$ and $CD8^+$ T cells elicited by DNA or recombinant viral vaccines and modulate their functional properties have been devised, such as strategies based on co-expression of cytokine and chemokine factors, costimulatory molecules, linkage to molecular adjuvants and antigen presenting cell-targeting ligands, modifications of protein sequences enhancing their immunogenicity, and design of poly-epitope constructs (Liu et al., 2006b). Infection of new world non-human primates with pathogenic SIV isolates such as SIV_{MAC239} or SIV_{MAC251} is the only available model for the testing of protective efficacy of vaccine candidates relevant to HIV-1 infection in humans. However, high cost and ethical issues bar large scale pre-testing of vaccines' immunogenicity in primates. Every effort should be taken to limit the size of experiments in non-human primates to the necessary minimum. Thus, small animal model is needed to test the immunogenicity and select the most

* Corresponding author at: Department of Pathology, University of Alabama at Birmingham, 845 19th Street South, BBRB 730, Birmingham, AL 35294-2170, USA. Tel.: +1 205 975 7079; fax: +1 205 975 2149.
E-mail address: zhel@uab.edu (Z. Hel).

0166-0934/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jviromet.2009.01.019

promising SIV vaccine candidates in pre-clinical trials. Although several CD8+ T cell epitopes in SIV Env and Gag protein recognized in mice have been defined, no study has systematically mapped CD4+ T cell epitopes in mice immunized with SIV Gag, a basic component of most SIV and simian–human immunodeficiency virus (SHIV) vaccine candidates. In this report, a detailed screening strategy is employed to map the available CD4+ and CD8+ T cell epitopes recognized in the two most common mouse strains, C57Bl/6 (B6) and Balb/c.

2. Materials and methods

2.1. Mice

Female C57Bl/6 and Balb/c mice, 6–8 weeks old, were obtained from the Jackson Laboratory (Bar Harbor, ME). All animal care and procedures conformed to the UAB Institutional Animals Care and Use Committee guidelines and requirements. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

2.2. Reagents

Gene encoding SIV Gag based on the sequence of SIV_{MAC239} isolate (accession nos. M19499, M15897, M16125, M24614, and AAB59905) was generously provided by Dr. G. Pavlakis. To optimize for mammalian expression, the previously identified *gag* inhibitory sequences (INS) were mutated by introducing multiple silent point mutations not affecting the encoded protein precursor, as previously described for HIV-1 *gag* (Qiu et al., 1999; Hel et al., 2001). DNA fragments encoding SIV Gag and sequence-optimized luciferase gene were cloned into an expression vector phCMV-1 encoding kanamycin resistance (Gene Therapy Systems, San Diego, CA). A set of 123 peptides (15-mers overlapping by 11 amino-acids) covering the entire SIV Gag protein sequence was obtained from the NIH AIDS Research & Reference Reagents program. All other peptides were synthesized by Genemed Synthesis, Inc. (South Francisco,

Table 1
Example of a peptide pool array.

		0.79	*0.2*	*.02*	*.02*	*.04*	*0.07*	*1.2*	*0.03*
		C9	**C10**	**C11**	**C12**	**C13**	**C14**	**C15**	**C16**
0.02	**R9**	63	65	67	69	71	73	75	77
1.16	**R10**	79	81	83	85	87	89	91	93
0.04	**R11**	95	97	99	101	103	105	107	109
0.06	**R12**	111	113	115	117	119	121	123	64
0.87	**R13**	66	68	70	72	74	76	78	80
0.09	**R14**	82	84	86	88	90	92	94	96
0.23	**R15**	98	100	102	104	106	108	110	112
0.04	**R16**	114	116	118	120	122	123		

Rows and columns labeled by bold numbers represent individual peptide pools; numbers in the table represent the sequential numbers of 15-meric peptides derived from SIV Gag protein included in each pool (6–8 peptides per pool, 123 peptides total). Each peptide is present in one horizontal and one vertical peptide pool. Numbers in italics on top and left side of the table represent the percentages of IFN-γ producing cells of total CD8+ cells determined in an assay using the corresponding peptide pool. Overlapping peptides 79 (308_QL15; present in pools R10 and C9) and 78 (313_AA15; present in pools R13 and C15) contain the immunodominant epitope 312_AL11 (see Fig. 1B).

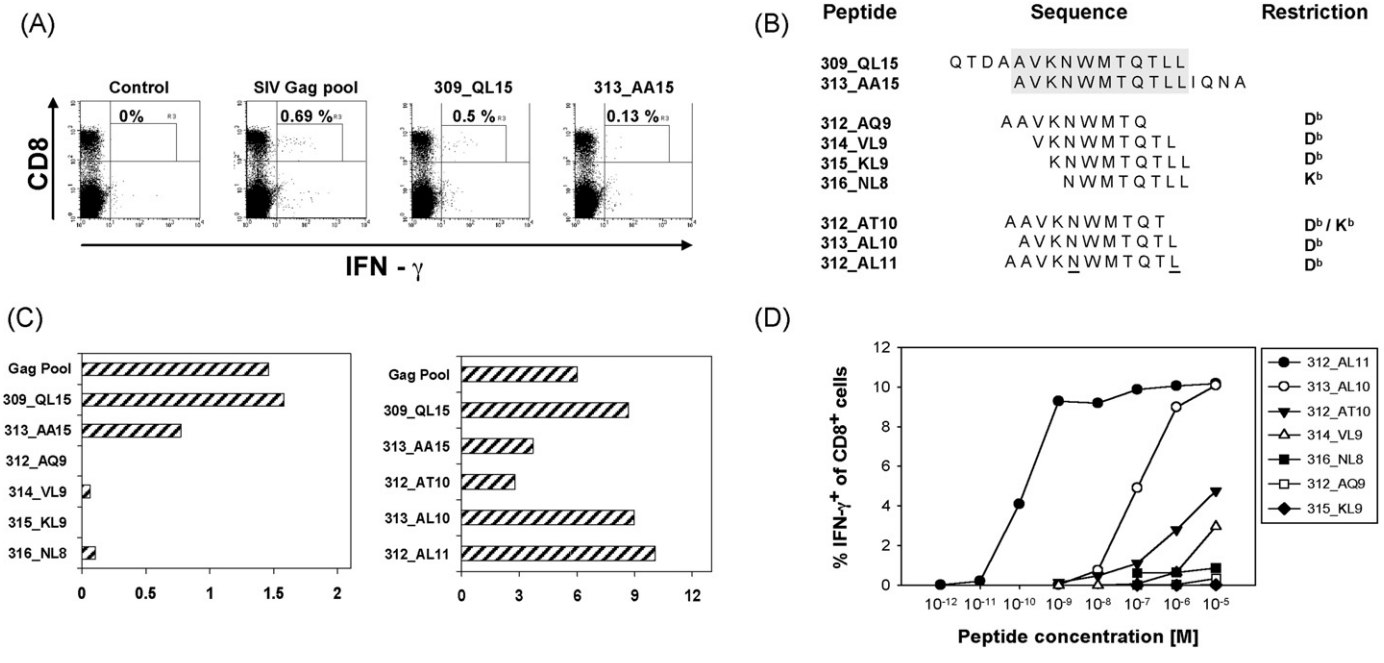

Fig. 1. Precise mapping of the dominant H-2^b-restricted SIV Gag epitope recognized in B6 mice. A: B6 mice were immunized once with 50 μg of plasmid expressing SIV Gag. Splenocytes were harvested and incubated with a pool of overlapping peptides covering the entire SIV Gag protein (123 peptides) or with individual peptides as indicated and the percentage of IFN-γ-producing cells of total CD8+ cells was determined. Each peptide is labeled using a position of the first amino acid in the Gag sequence, single-letter codes of the first and last amino acids, and the total number of amino acids it contains. B: Sequences of peptides used for the precise mapping of the dominant H-2^b-restricted epitope. Underlined amino acids represent putative anchor residues. C: Splenocytes from SIV Gag-immunized B6 mice were incubated with individual peptides at 1 μM concentration and the percentage of IFN-γ-producing cells was determined. Representative data from 2 of 4 similar experiments are shown. D: Splenocytes from SIV Gag-immunized mice were incubated with individual peptides at increasing concentrations and the percentage of IFN-γ-producing cells was determined. Data from 1 of 2 similar experiments are shown.