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硕士 学位 论文

人免疫缺陷病毒包膜蛋白第三可变区 V3 融合蛋白的免疫原性分析及中和单抗的筛选

Immunogenicity Assay on Fusion  
Proteins Harboring Envelope V3 Loop of Human  
Immunodeficiency Virus and Subsequent Neutralizing  
Monoclonal Antibody Screening

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## 摘要

获得性免疫缺陷综合征（AIDS）是一种以机体免疫系统严重损害为主要特征的传染性疾病，人类免疫缺陷病毒 1 型（HIV-1）感染是 AIDS 的基础。2015 年为止，HIV 全球感染人数达到 3670 万，严重危害人类健康。迄今为止，仍无保护性疫苗或能彻底清除 HIV 病毒的药物出现。因此研究安全有效的疫苗和抗病毒药物对于预防和治疗 HIV 有重大意义。

自 2009 年以来，有大量强效广谱中和抗体和相应表位被报道，动物模型以及 RV144 疫苗的保护效果都证明诱导机体产生广谱中和抗体是疫苗设计的关键。HIV-1 包膜糖蛋白 gp120 第三可变区（V3）对病毒的感染至关重要，在前期的研究中，我们通过 HIV 病毒免疫筛选到了特异针对 HIV 毒株 NL4-3 V3 表位的中和抗体，本研究将 V3 中和表位展示在 CRM197-A 和 HPV-VLP 上，评估 V3 中和表位的免疫原性和研究开发表位疫苗的可能性，进而为预防性疫苗和治疗性抗体的研究提供基础。

在本研究中，我们将不同长度，不同型别的 V3 中和表位展示在 CRM197-A 和 HPV-VLP 上融合表达，通过中和实验和酶联免疫吸附实验（ELISA）评估小鼠体液免疫效果。基于实验室成熟的小鼠单克隆抗体筛选平台，利用基于免疫斑点印迹法（ELISPOT）的 HIV 中和抗体筛选平台筛选单克隆抗体，通过中和实验和 ELISA 对单抗进行性质分析。通过对小鼠血清监测结果和单抗性质分析，综合评价 V3 中和表位的免疫原性，进一步探索开发表位疫苗的可能性。

在研究过程中，将不同长度，不同型别的 V3 中和表位融合 CRM197-A 和 HPV-VLP 为免疫原，血清监测结果显示 CRM197-A-NL4-3-299-328 和 HPV-NL4-3-296-311 融合蛋白免疫血清对 HIV<sub>NL4-3</sub> 有中和效果。CRM197-A-NL4-3-299-328 融合蛋白免疫小鼠筛选到 8 株特异中和 HIV<sub>NL4-3</sub> 的中和单抗，其 IC<sub>50</sub> 大部分低于 0.02 μg/mL，是强效的型特异性中和单抗。HPV-NL4-3-296-311 融合蛋白免疫小鼠筛选到 20 株中和单抗，其中和能力不一，大部分单抗能够结合不同型别的 HIV 包膜蛋白 gp120，具有交叉亲和的效果。其中单抗 4H4 具有交叉中和的效

果，对实验室现有毒株 HIV<sub>NL4-3</sub>, HIV<sub>89.6</sub>, HIV<sub>MJ4</sub>, HIV<sub>94</sub> 均有中和效果，IC<sub>50</sub> 为 12-15 μg/mL。

综上，本研究中，V3 中和表位融合蛋白刺激小鼠产生高中和效价，筛选到了型特异性强效中和抗体，同时也有交叉中和抗体的产生，表明 V3 中和表位融合蛋白具有强的免疫原性，有诱导产生广谱中和抗体的潜力，为后续诱导广谱中和抗体抗原的设计和表位疫苗的研究提供基础。

**关键词：**人免疫缺陷病毒；中和表位；表位展示；交叉中和抗体；包膜蛋白 V3 区域

## Abstract

Acquired immunodeficiency syndrome (AIDS) is an infectious disease characterized by severe systemic immune system damage. Human immunodeficiency virus type 1 (HIV-1) infection is the archcriminal of AIDS. By 2015, the number of HIV global infectious person reached 36.7 million, seriously endangering human health. So far, there is no protective vaccine or drugs that can completely remove the HIV virus. Therefore, the study of safe and effective vaccines and antiviral drugs for the prevention and treatment of HIV is of great significance.

Since 2009, a large number of potent and broadly neutralizing antibodies and corresponding epitopes have been reported, animal models and RV144 vaccine protection effects have shown that induction of broadly neutralizing antibodies is the key to vaccine design. HIV-1 envelope glycoprotein gp120 the third variable region (V3) is essential for viral infection. In our previous study, we immunized mice with virus and screened antibody against HIV strain NL4-3 V3 epitope, the V3 neutralizing epitope was shown on CRM197-A and HPV-VLP to assess the immunogenicity of V3 neutralizing epitopes and the potential for research and development of epitope vaccines, and then provide the basis for prophylactic and therapeutic antibody research.

In this study, we showed different fusion protein of different length and different types of V3 in CRM197-A and HPV-VLP, and evaluated the humoral immunity of mice by neutralization experiment and enzyme-linked immunosorbent assay (ELISA) effect. Based on the mouse monoclonal antibody screening platform, the monoclonal antibody was screened using the immunoblot blot (ELISPOT), based HIV neutralizing antibody screening platform. The characterization of the monoclonal antibody were analyzed by neutralization assay and ELISA. Through the analysis of mouse serum and monoclonal antibody characterization analysis, the immunogenicity of V3 neutralizing epitope was comprehensively evaluated, and the possibility of developing epitope vaccine was further explored.

## Abstract

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In the course of the study, CRM197-A and HPV-VLP were fused with different length and different types of V3 neutralization epitopes. Serum monitoring results showed that CRM197-A-NL4-3-299-328 and HPV-NL4-3-296-311 fusion protein immunized serum has neutralizing effect on HIV<sub>NL4-3</sub>. CRM197-A-NL4-3-299-328 fusion protein was screened 8 neutralizing monoclonal antibodies with specific neutralizing HIV<sub>NL4-3</sub>, most of which IC<sub>50</sub> was less than 0.02 µg/mL, which was potent monoclonal antibody. HPV-NL4-3-296-311 fusion protein was screened 20 strains of neutralizing monoclonal antibody with different capacities. Most of the monoclonal antibodies were able to bind to different types of HIV envelope protein gp120, which with cross-reactivity. The mAb 4H4 could neutralize the laboratory existing strains HIV<sub>NL4-3</sub>, HIV<sub>89.6</sub>, HIV<sub>MJ4</sub>, HIV<sub>94</sub>, and IC<sub>50</sub> of HIV<sub>NL4-3</sub>, HIV<sub>89.6</sub>, HIV<sub>MJ4</sub> and HIV<sub>94</sub> were 12-15 µg/mL.

In conclusion, in this study, V3 neutralizing epitope fusion protein stimulated mice to produce potency antibody, and screened for type-specific potent neutralizing antibodies, and also produced cross-neutralizing antibodies, suggesting that V3 neutralizes epitope fusion has strong immunogenicity and has the potential to induce broadly neutralizing antibodies, which providing basis for further studies on the design of broadly neutralizing antibody antigens and epitope vaccines.

**Keywords:** Human Immunodeficiency Virus; neutralizing epitope; fusion protein; cross neutralizing antibodies; v3 loop

## Abbreviations

AIDS Acquired Immune Deficiency Syndrome , 获得性免疫缺陷综合征

HIV-1 Human immunodeficiency virus type 1 , 人类免疫缺陷病毒 1 型

SIV Simian immunodeficiency virus , 猴免疫缺陷病毒

UNAIDS The United Nations Joint Programme on HIV/AIDS , 联合国艾滋病规划署

WHO World Health Organization , 世界卫生组织

ART Antiretroviral therapy , 抗病毒治疗

bNAbs broad neutralization antibodies , 广谱中和抗体

CCR5 C-C chemokine receptor 5 ( $\beta$ -chemokines) , 趋化因子受体

CXCR4 C-X-C chemokine receptor 4 ( $\alpha$ -chemokines) , 趋化因子受体

env Envelope gene , 包膜蛋白

gag Group specific antigen gene , 组织特异抗原基因

pol Polymerase gene , 聚合酶

MA Matrix protein p17 , 基质蛋白 p17

CA Capsid protein p24 , 衣壳蛋白

NC Nucleocapsid protein p7 , 核壳体蛋白

IN integrase , 整合酶

nef Negative effector gene , 负调控因子

rev Regulator of expression of viral proteins gene , 病毒表达调控蛋白

tat Trans-activator of transcription gene , 转录活性因子

vif Virion infectivity factor gene , 病毒感染因子

vpr Viral protein, regulatory gene , 病毒蛋白 R

vpu Viral protein, unknown gene , 病毒蛋白 U

LTR Long terminal repeat , 长末端重复序列

DNA Deoxyribonucleic acid , 脱氧核糖核酸

cDNA complimentary DNA , 互补脱氧核糖核酸

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