加载抗原肽的单体及其四聚体制备和鉴定

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摘要
cell-mediated lymphocytes \( (E5F) \) in controlling viral and bacterial infections and tumor processes play a very important role. After initial sensitization, the antigen-specific T cell population expands and acquires cytotoxic activity and effector functions. To better understand the antigen-specific \( E5F \) response, it is necessary to carefully analyze the number and function of the antigen-specific \( E5F \) cells; however, these \( E5F \) cells are often overwhelmed by other cells. The detection of antigen-specific \( E5F \) cells requires in vitro culturing and repeated stimulation, which are prone to introduce bias. For example, limiting dilution analysis \( (FT4) \) is a standard method for measuring \( E5F \) frequency, but there is evidence that this method underestimates the number of \( E5F \) cells. Other methods, such as flow cytometry with intracellular cytokine staining, also have similar drawbacks. Therefore, there is a need for a more direct method to detect antigen-specific \( E5F \) cells.

Cell activation, proliferation, and effector functions are induced through the \( E5F \) receptor and the peptide-major histocompatibility complex \( (MHC) \), which are expressed on antigen-presenting cells. Therefore, the soluble MHC for \( E5F \) staining is an attractive strategy, but due to the low affinity between MHC and \( E5F \) receptors, this method has never been successful. This obstacle has been overcome by MHC tetramer technology \( (T4) \), which allows the direct detection of antigen-specific \( E5F \) cells using flow cytometry.
备过程

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1

1.1

1.1.1

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1.2

1.2.1

1.2.2

1.2.3

1.2.4
1.2.5 HLA-A2 NLV  A2-NLV  A2-NLV  A2-NLV  2 mg NLV  200 mL  100 mmol/L Tris-HCl pH 8.0  400 mmol/L L-arginine  2 mmol/L EDTA  5 mmol/L reduced glutathione  0.5 mmol/L oxidized glutathione  0.2 mmol/L phenylmethylsulfonyl fluoride  PMSF  6 mg A2  0.5 mL  3 mol/L Guanidine-HCl  pH 4.2  10 mmol/L sodium acetate  10 mmol/L EDTA  26°C  4°C  5 mg β-m  4°C  3 d  Amicon  10 000 200 mL  10 mmol/L Tris-HCl pH 8.0  0.2 mmol/L PMSF  3 h  4°C  10 min  4°C  10 min  4°C

1.2.6 A2-NLV  A2-NLV  A2-NLV  30°C  40 min  0.2 mmol/L PMSF  0.2 mmol/L PMSF  10 mmol/L Tris-HCl buffer pH 8.0  0.2 mmol/L PMSF  Q-Sepharose  0  300 mmol/L NaCl  Pharmacia GradiFrac System  PAGE  Amicon Ultra-4 MWC010000  300 μL  4 mL  PBS  0.2 mmol/L PMSF  2 mmol/L EDTA  300 μL  4°C

1.2.7 A2-NLV  A2-NLV  A2-NLV  4:0.8  5 10 min  SDS-PAGE  Laemmli  5%  12%  15%  R-250

1.2.8 SDS-PAGE  Laemmli  5%  12%  15%  R-250

1.2.9 HLA-A2 NLV  A2-NLV  40 yrs  3 mL  10%  50 μL PBS-2% FCS  CD3-FITC CD8-CyChrome  10 μIU  4 μg A2-NLV Tetramer Streptavidin-PET  1 h  100 bp DNA  8  1A lane 6 1B lane 1 3 2 1A lane 6 1B lane 4 1 1B lane 4 1B lane 4 ATG  pET-2A

2.1 HLA-A * 0201  IMGT/HLA  HLA-A * 0201  PB-MC  RNA  cDNA  PCR  DNA  1100 bp DNA  1A lanes 2 4  DNA  pET-3c  DH5a  8  8  1A lane 6 1B lane 1 3 2 1A lane 6 1B lane 4

2.2 BSP  HLA-A * 0201  HLA-A2  A2  Alman  PCR  HLA-A2  1-275 DNA  BSP  Gly-Ser  HLA-A2  900 bp  1B lane 4 1B lane 4 1B lane 4 ATG  pET-2A

2.3 A2  β-m  E. coli  β-m  E. coli  BL2  DE3 pLysS  30%  A2  E. coli  BL2  DE3  pET-2A  A2  E. coli  BL2  DE3  R-250
2.4 A2-NLV

A2-NLV

\[ \text{A2-NLV} \]

15% SDS-PAGE

A2-NLV

SDS-PAGE

12 kD

\( \beta_2m \)

3 lane 2

A

GILGFVFTL

A2-NLV

3 lane 3

Q-Sepharose

NaCl

\( \beta_2m \)

\( \text{HLA-A } \times 201 \)

18

\( \beta_2m \)

3 lane 4

\( \text{I} \)

11

21-25

\( \text{HLA-A } \times 201 \)

2-NLV

SDS-PAGE

12 kD

\( \beta_2m \)

3 lane 5-9

\( \text{I} \)

260 nm

280 nm

A2-NLV

22

23

24

4 mg/mL

Fig. 2 SDS-PAGE analysis of extracellular domain

of HLA-A*0201 A2 heavy chain expressed

e in E. coli strain BL21

1 MW markers 2 refolded A2-NLV monomer 3 biotinylated A2-NLV

monomer 4-10 fractions no. 18 21 22 23 24 25

2 see Fig. 4 respectively. HC heavy chain \( \beta_2m \) \( \beta_2m \)-microglobulin

2.5 A2-NLV

Streptavidin-PE

SDS-PAGE

35 kD

12 kD

\( \beta_2m \)

5 lane 5

\( \text{I} \)

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Fig. 3 SDS-PAGE analysis of HLA-A*0201

NLA A2-NLV monomer

HLA-A*0201

Bl21

DE3

DE3

Fig. 1 Restriction enzyme digestion of the recombinant

to containing HLA-A*0201 A2 gene and the expression

vector for A2 heavy chain fused with BSP sequence

at its carboxyl terminus

A 1 1 200 bp DNA ladder 2 2 3 4 RT-PCR products from 3 donors

5 pET-3cf Ndel+BamHI; 7 8 vector for A2 heavy chain gene Ndel+BamHI

B 1 2 200 bp DNA ladder 2 PCR product for extracellular

domain of A2 heavy chain 4 expression vector for A2 heavy chain gene

Ndel+BamHI 5 pET-3cf Ndel+BamHI.
The column was equilibrated with 10 mmol/L Tris-HCl pH 8.0. The sample dialyzed against the same buffer was loaded onto the column and eluted with 0–300 mmol/L NaCl linear gradient at a flow rate of 0.8 ml/min. Fractions of 3 ml were collected. Fractions 22–24 containing pure A2-NLV were pooled indicated by bars.

Fig. 6 Flow cytometry analysis of the staining of HCMV-specific CTL with HLA-A * 0201 NLVVMATV hexamer. PBMC from an HLA-A2 donor was stained with HLA-A * 0201 pp65_{555–563} tetramer. Data represent the percentages of A2-NLV tetramer positive CTL within total T cells gated on CD3+ cells. A ■ Control ■ Streptavidin-PE B ■ A2-NLV Tetramer.

3 Altman (1996) TCR
d

pMHC
d

BSP
d

Lys
d

MHC I
d

HLA-A2
d

15
d

BirA
d

Avidin
d

Streptavidin
d

HPLC
d

FPLC
d

Garbozzi
d

HCM

检测到较高水平的特异性分比为免疫研究建立必要的技术平台，而且该四聚体本身及移植后这些特异性
的最高频等位基因，在中国人中约有强烈相关性。人群的分布不一，其中
期何贤辉等：加载的频率可能高于以往的估计，这在一定程度上说明中国人中
限制性的免疫应答中表现出明显表
特表
，其中
疾病，而移植物中
限制性的免疫应答有
这在一定程度上说明中国人中
疾病的灵敏工具，对临床实践有重要
，利用
四聚体可以用于
四聚体可以用于
，其中
！JG %)* b?LAP 0OOa
！JG %)* b?LAP 0OOa

REFERENCES
Preparation and Characterization of HLA-A*0201 Monomer and Tetramer Loaded with HCMV Antigenic Peptide

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Abstract Quantification of cytotoxic T lymphocytes (CTL) is extremely important due to the pivotal role they play in controlling pathogen infection and anti-tumor actions. Previously used methods for detecting specific CTL are usually indirect. In recent years, tetramer technology has been developed to directly visualize antigen-specific CTL efficiently and become the critical approach in studying T cell immune responses. A simplified procedure for preparing tetramers is reported here in this paper and a tetramer loaded with human cytomegalovirus (HCMV) peptide was successfully obtained using this procedure which possessed binding activity with specific CTL. The heavy chain of HLA-A*0201 gene was cloned by RT-PCR from HLA-A2⁺ donor. An expression vector encoding the extracellular domain of HLA-A*0201 heavy chain A2 was fused with a BirA substrate peptide at its carboxyl terminus was constructed by PCR with cloned A2 gene as the template. The A2 heavy chain was expressed in Escherichia coli mostly in the form of inclusion body and purified by washing inclusion body. The monomer of soluble A2 loaded with peptide was reconstructed by dilution from the heavy chain in the presence of light chain β₂-microglobulin and HLA-A2 restricted HCMV pp65,050-063 peptide NLVPMTAV[\text{NLV}]. Refolded A2-NLV monomer was biotinylated with a commercial BirA and purified by low pressure anion exchange chromatography on a Q-Sepharose fast flow column. The tetramer was then formed by mixing A2-NLV monomer with streptavidin-PE in a ratio of 4:1, 8 leading to more than 85% multiplicity as revealed by SDS-PAGE under non-reducing conditions without boiling the sample. Flow cytometry analysis indicated that this tetramer could bind to specific CTL from HLA-A2⁺ donor. In conclusion, a simplified procedure is established to prepare HLA-A2 tetramer which may not only facilitate the application of tetramer technology for studying specific T lymphocyte immune response but A2-NLV itself be applied clinically to monitor CMV-specific CTL in stem cell and organ transplantation.

Key words human leukocyte antigen tetramer cytotoxic T lymphocyte inclusion body human cytomegalovirus

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