Enhanced in vivo CTL activity by newly synthesized micelles loaded with Trp2 peptide

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ABSTRACT SUMMARY

To develop a safe and efficient vaccine delivery system for melanoma immunotherapy, in this study, hydrophobic antigen peptide Trp2 was loaded in newly chemically synthesized Polyethyleneimine(2k)-stearic acid (PSA) micelles. Our results demonstrated PSA had a powerful adjuvant activity in a dose-dependent manner, and PSA-Trp2 micelles could induce significant Trp2-specific CTL activity in vaccinated mice.

INTRODUCTION

In order to develop new, safe and efficient tumor vaccine delivery systems providing persistent term protection against tumor growth or metastasis1, not antigen delivery vector but also adjuvant that promote DC maturation and antigen presentation has caught more increased concerns. It has been demonstrated that after s.c. administration to mice, nanoparticles of a small size(20–45 nm) could specifically target DCs in lymph nodes and facilitate nanoparticles uptake by DCs.2 Based on this, although some polymer nanogels emerged as negative charged OVA antigen delivery vehicles by chemical conjugation or physical absorption, it was hard to be applied in tumor peptide vaccine delivery since tumor peptide bears ignorable charge.

Alternatively, in our study, to construct a hydrophobic Trp2 peptide delivery system for melanoma immunotherapy, Polyethyleneimine 2k-stearic acid (PSA) micelles with a small size was first designed for function both as an antigen delivery vector and a new adjuvant in peptide vaccine delivery system. And PSA loaded Trp2 micelles (PSA-Trp2) were formed by dialysis method. We hypothesized PSA could facilitate the presentation of Trp2180–188 (H-SVYDFFVWL–OH) by DC cells to immune cells and induce an efficiently Trp2-specific CTL activity after s.c administration into mice.

EXPERIMENTAL METHODS

The size distribution and zeta potential of PSA-Trp2 micelles were measured by photon correlation spectroscopy (PCS) using a Zeta sizer Nano ZS90 (Malvern instruments Ltd., UK). The morphology of PSA loaded Trp2 micelles was observed using a Transmission electron Submitted to microscopy (TEM) instrument (H-600, Hitachi, Japan).

The expression level of CD80 on BMDC was investigated using a Beckman Coulter Quanta SC cytometer(Beckman Coulter, CA, USA). On the 6th day of mouse BMDC maintained in specific culture medium, PSA of a different dose was added to and incubated with BMDC for 24h at 37°C. Then cells were marked with FITC-anti-mouse CD80 monoclonal antibodies and flow cytometry was performed to evaluate the CD80 expression level on BMDC.

For in vivo CTL killing assay, female C57BL/6 mice were s.c. immunized with Trp2(100μg per mice) or PSA-Trp2 micelles. 14 days later, splenocytes isolated from naïve mice were divided equally into two aliquots. One was pulsed with 2 M Trp2 peptide and the other with medium for 2 h at 37 °C. Next, the peptide-pulsed and medium-treated cells were respectively marked with a high CFSE concentration (4 M) and a low one (0.4 M). The two aliquots (5x10^7/mL) were mixed together, and 200μL was given to naïve or immunized mice. 18 h later, the spleen mononuclear cells from recipient mice were analyzed by flow cytometry. Specific lysis percentage was evaluated as special formula.

RESULTS AND DISCUSSION

The optimal ratio of PSA and Trp2 was determined as 4:1(w/w) (Data not shown), in which case, the encapsulation efficiency(EE%) was 99.54%. The TEM image showed PSA-Trp2 micelles at optimal ratio were well-distributed without any aggregation and the size was about 30nm (Figure 1A). This was consistent with the result of photon correlation spectroscopy (28.7±8.2nm)
and the zeta potential of the sample in saline was 17.3±5.8mV.

Figure 1. Characterization of PSA-Trp2 micelles. A) TEM image of PSA-Trp2 micelles. B) Size distribution of micelles measured by photon correlation spectroscopy.

Adjuvant could promote BMDC maturation, on which, co-stimulators such as CD40, CD80, CD83 and so on were expressed. In our study, after BMDC was stimulated with PSA for 24h at 37°C, the expression level of CD80 was evaluated on flow cytometer after cells were marked with monoclonal antibody FITC-anti-CD80. As shown in the Figure 2, the expression level of CD80 depended on the concentration of PSA micelles. When it reached to 50 μg/mL, nearly 100% of the positive cell expression efficiency achieved. Simultaneously, the expression level of CD83, CD86 were also investigated. The results indicated that PSA micelles had a strong adjuvant activity since they significantly improved BMDC maturation in a dose-dependent manner.

Figure 2. Quantitative evaluation of positive efficiency (the gray) and mean fluorescence intensity (the red) of CD 80 expression on BMDC cells after the cells was stimulated with PSA micelles of different concentration of 2, 10, 50 and 100 μg/mL (The labels as PSA-2, PSA-10, PSA-50, PSA-100). Data are represented as mean ±SD (n=3). **p < 0.05, ***p < 0.001.

The vivo CTL assays showed free Trp2, Trp2 mixed with PSA micelles (PSA+Trp2) or Trp2 loaded PSA micelles (PSA-Trp2) could induce the generation of Trp2-specific CTL. Compared with mice group treated with the mixture group, Trp2 loaded PSA micelles significantly augmented CTL activity with a specific lysis percentage of 51%(p < 0.001). (Figure 3)

Figure 3. In vivo CTL assay of C57BL/6 mice immunized with saline(control), PSA, free Trp2, Trp2 mixed with PSA micelles (PSA+Trp2) or Trp2 loaded PSA micelles (PSA-Trp2). Data are represented as mean ±SD (n=5), *p <0.05, ***p < 0.001.

CONCLUSION

In conclusion, we developed a new tumor peptide vaccine system, making antigen and adjuvant co-delivery for melanoma immunotherapy. The system not only facilitated the administration of hydrophobic antigen peptide Trp2, but also significantly induced the generation of CTL response.

REFERENCES