Long-term protection against B16F1 melanoma upon vaccination with tumor cell lysate combined with LTX-315 as a novel adjuvant

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Background

Here, we demonstrate the potency of LTX-315 as a novel adjuvant in combination with B16F1 tumor cell lysate (TCL) in a prophylactic model. LTX-315 is a synthetic membrane active host-defence peptide that induces im-munogenic cell death which leads to local inflammation and activation at the injection site (Figure 1 A and B). This mechanism is important to obtain strong vaccine-specific immune responses.

In addition, these class of molecules may have a direct modulatory effect on the immune system, in particular professional antigen presenting cells (APCs). Preliminary data demonstrated that LTX-315 enhances the uptake of antigen by human dendritic cells (data not shown). See also abstract/poster no. 474 for an elaborated description of the mechanisms of LTX-315.

Aim

To demonstrate the potency of LTX-315 as a novel adjuvant in B16F1-melanoma model.

Methods

CS7BL/6N mice received 80 µg/mL LTX-315 i.d. Skin biopsies were taken at selected time points post injection (hours) 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, 168. The samples were analyzed for the degree of necrosis by H&E staining, and the samples were stained with CD45-positive antibodies. In addition, CD45-positive cells were analyzed via immunohistochemistry of the influx of CD45-positive cells.

Vaccine antigen:

B16F1 tumor cell lysate (TCL) established by 5 repeated freeze and thaw cycles, -20°C and 37 °C. TCL consisted of 3x10⁶ B16F1 cells.

Adjuvant: LTX-315 60 µg/mL.

CS7BL/6N mice were vaccinated s.c once a week for 4 weeks with B16F1 TCL (3x10⁶ B16F1 cells/cycle dose) combined with LTX-315 as adjuvant. LTX-315 were injected either 2 hours prior to, simultaneously with or 2 hours after TCL injection. The animals were challenged with 3x10⁶ B16F1 s.c. two weeks after the last vaccination. Tumor protected mice were rechallenged 10 weeks after the primary tumor challenge. To demonstrate long-term protection to B16F1, animals that survived the previous tumor rechallenge, received a second tumor rechallenge at week 45 (Figure 2).

Figure 1

A) Preliminary data demonstrating survival benefit in animals vaccinated with TCL combined with LTX-315 as adjuvant. There were no substantial differences between the timing of LTX-315 injection in combination with B16F1 TCL, after tumor challenge.

B) The vaccination experiment was repeated with 10 animals/group. Tumor challenge 2 weeks after the last vaccination demonstrated survival benefit of LTX-315/TCL vaccinated groups. However, the results showed a trend that giving LTX-315 2 hours before TCL (8/10 surviving animals) may be more beneficial than giving this adjuvant simultaneously or 2 hours after TCL injection.

Figure 2

The overall kinetics in the skin presented as the degree of necrosis and inflammation throughout the study period.

Figure 3

A) Pilot study 6 animals/group

B) Repeated study with 10 animals/group

A) Animals protected against tumor growth from the repeated experiment, had established long-term protection against B16F1 up to 45 weeks post primary tumor challenge. A second rechallenge of the tumor protected animals displayed a similar protection pattern. This indicates that a strong immunological memory against tumor antigen. In addition, we have shown that LTX-315 is a potent adjuvant with tumor derived peptide antigens in B16F1-melanoma model.

The ability of LTX-315 to induce an inflammatory response at the injection site in addition to direct immunomodulatory effects, may reflect the adjuvant potency of the molecule to enhance the vaccination efficacy. LTX-315 may be represented as the next generation adjuvant with a potential be combined with therapeutic cancer vaccines.

Conclusions

The present study demonstrates that LTX-315 is a potent adjuvant when combined with tumor cell lysate in the B16F1-melanoma model.

LTX-315 is currently being investigated as an adjuvant in therapeutic B16F1 and B16F10 melanoma models utilizing TCL as the source of tumor antigen. In addition, we have demonstrated that LTX-315 may be more beneficial than giving this adjuvant simultaneously or 2 hours after TCL injection.