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 immunogenic 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

C57BL/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 inflammation by immunohistochemical analysis of the influx of CD45-postive cells.

Vaccine antigen:

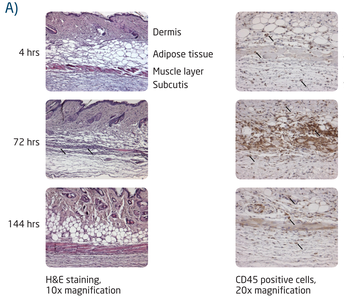
- B16F1 tumor cell lysate (TCL) established by 5 repeated freeze and thaw cycles (-70°C and 37 °C). TCL consisted of 3x10^6 B16F1 cells.

- Adjuvant: LTX-315 60 µg/mL.

C57BL/6N mice were vaccinated s.c once a week for 4 weeks with B16F1 TCL (3x10^6 B16F1 cells/lysate dose) combined with LTX-315 as adjuvant. LTX-315 were injected either 2 hours prior to, simultanously with or 2 hours after TCL injection. The animals were challenged with 5x10^4 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 had survived the previous tumor rechallenge, received a second tumor rechallenge at week 45 (Figure 2).

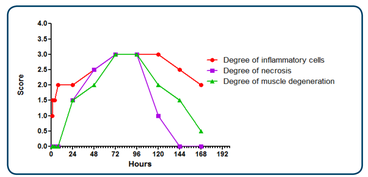
Figure 1

A)



A)
H&E and CD45 staining of three selected time points. Complete healing of the tissue at the injection site one week after the injection.

B)



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

Figure 2

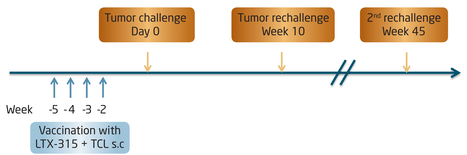
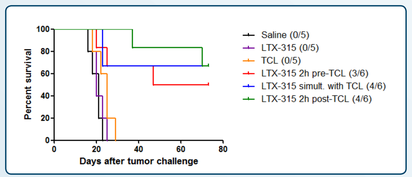


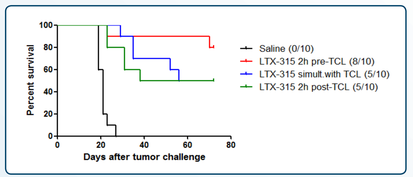
Figure 3

A) Pilot study 6 animals/group



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.

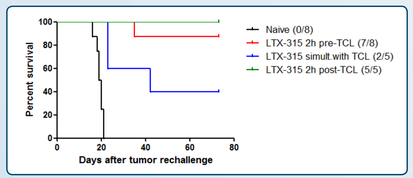
B) Repeated study with 10 animals/group



B) The vaccination experiment was repeated with 10 animals/group. Tumor challenge 2 weeks after the last vaccination demonstrated survival benefit in all 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 simultanously or 2 hours after TCL injection.

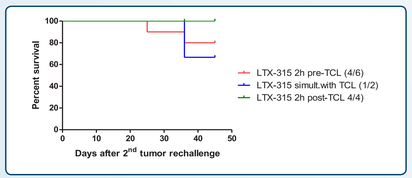
Figure 4

A) Rechallenge of tumor protected animals



A) Animals protected against tumor growth from the repeated experiment, were rechallenged 10 weeks after the primary challenge. Animals that received LTX-315 simultanously with TCL, showed to have less survival benefit upon rechallenge than animals receiving LTX-315 2 hours before or after injection of TCL. Taking all the vaccinated groups together, 14/18 animals had developed long-term protection against B16F1 up to 45 weeks post primary tumor challenge.

B) 2nd rechallenge, 45 weeks post-primary challenge



B) To demonstrate wether the long-term protection still was durable one year after the primary tumor challenge, a 2nd tumor rechallenge was performed with B16F1 cells at week 45. Two of the 14 animals had to be euthinized before tumor rechallenge due to health issues. Twelve animals were s.c challenged with B16F1 cells and 9 were tumor free >40 days post-2nd rechallenge.

Conclusions

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

In total, 14/18 animals had established long-term protection to B16F1 cells upon rechallenge 10 weeks after the primary tumor challenge. A second rechallenge of the surviving animals 45 weeks post primary challenge displayed a similar protection pattern. This indicates that a strong immunological memory against B16F1-melanoma has been developed in 9/12 animals that received a second rechallenge approx. 1 year after vaccination with B16F1 TCL combined with LTX-315 as adjuvant.

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 initiated studies to combine LTX-315 as an adjuvant with tumor derived peptide antigens in B16F10-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.

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