Treatment of two dogs with mast cell tumors using autologous immunotherapy

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Cancer cells are genetically unstable and produce abnormal proteins which, for various reasons, are not eliminated by the immune system. Due to this instability, the abnormal proteins are not always the same from one patient to other and can change all along the natural history of the disease. This is the rationale for a customized immunotherapy in order to match the antigenic evolution of the tumor.
We used a protocol of autologous immunotherapy containing hydroxylapatite particles associated to autologous tumor proteins in order to treat two high grade mast cell tumors.

Materials and methods:

Vaccine:

The vaccine is made from a tumor biopsy.
It is composed of **heat shock proteins** for its antigenic part and of **hydroxylapatite** (HA) particles as adjuvant.
Heat shock proteins (HSPs) produced in large amounts by cancer cells are purified by adsorption chromatography on hydroxylapatite micro/nanoparticles *(Fig. 1)*.
HSPs are chaperone molecules produced when cells are submitted to a stress and aimed to stabilize the peptides that they chaperone during the period of stress. Purifying these molecules allows getting a fingerprint of the proteins synthesized by the cells. They also have a role in the presentation of their associated peptides by the presenting cells and thus in the immune survey of transformed cells.
Once loaded by these chaperone proteins extracted from a tumor biopsy, the HA particles are injected in the patient subcutaneous tissue. The nanoparticles are used as the solid phase of the chromatography and adjuvant of vaccination.
It was demonstrated that CD8 cells were cross-primed against the proteins carried by the nanoparticles (Fig. 2). These proteins were ligand of cd91 on the antigen presenting cells.

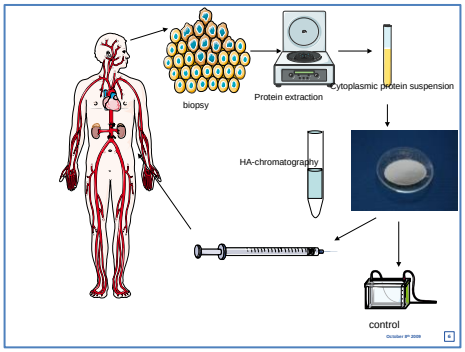

Fig. 1: the vaccine is made from a tumor biopsy by extraction of cytoplasmic proteins and adsorption on HA-powders through a chromatography columns

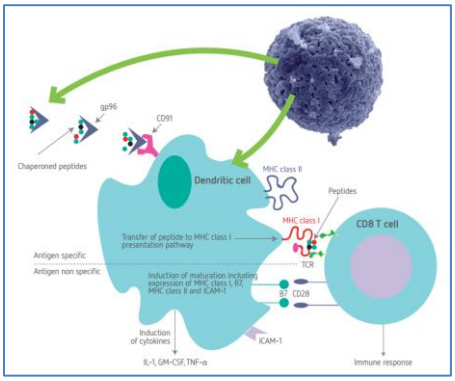

Fig. 2: the HSPs proteins carried by the HA-particles are ligand of cd91 on the APCs. They process their associated peptides at the surface of type I HLA proteins. HA-particles are activator of the inflammasome

Dogs selection:

Dog 1: a 13 year old dog (Weimaraner) suffering from a grade III mast cell tumor at the basis of the tail without any sign of dissemination was operated for the first time preserving cutaneous margins around the tumor (fig 3, 4).
Dog 2: A 11 years old dog (Labrador) suffering from a grade II mast cell tumor in 2 locations: periumbilical region (1x1,5cm) and neck (1,5x2cm). A surgery was performed on the neck lesion while the peri-umbilical tumor was not operated.
For both dogs the treatment schedule was at least one dosis /week for one month and one dosis / month for 4 months.

Results:

Dog 1: The tumor reoccurred once at 40 days after the first operation, then again at 80 and 140 days. The tumor was re-operated 48 hours after each reoccurrence.
At the second reoccurrence, the tumor was inflammatory. Indeed the cutaneous margins were not respected after the second operation. It was decided that the vaccination would be continued every week instead of the normal 8 dosis cycle.
After the fourth reoccurrence, the tumor disappeared totally and did not reoccur until the animal death one year later for a reason which was not related
Dog. 2: This dog received a complete cycle of autologous adaptive immunotherapy.
The peri- umbilical lesion shrank from three weeks after the first injection to six weeks when it disappears. He died 835 days after diagnostic without recurrence (natural death)

Fig. 3: Dog 1: initial tumor

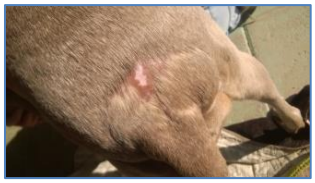

Fig. 4: Dog 1 tail basis at 9 months

Discussion and conclusions:

This kind of autologous immunotherapy can be used for the treatment of mast cell tumors. We did not see any secondary effects, the dogs keeping a normal activity during the treatment.
This is a simple way (the dosis preparation takes about one hour) to restore immunity against the tumor cells. Dog 1 showed that changing the vaccination schedule and increasing the frequency of vaccination could trigger an immunity even when there was a resistance

Fig. 5: Apavac kit as it is made commercially available in veterinary medicine