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What is the influence of vaccination's routes on the regression of tumors located at mucosal sites?

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Tumor-regressions following tumor-associated-antigen vaccination in animal models contrast with the limited clinical outcomes in cancer patients. Most animal studies however used subcutaneous-tumor-models and questions arise as whether these are relevant for tumors growing in mucosae; whether specific mucosal-homing instructions are required; and how this may be influenced by the tumor.

Persistent infection by oncogenic types of human papillomavirus (HPV) is the central etiological agent of several ano-genital cancers, including cervical cancer, the second cause of cancer death in women worldwide. Since carcinogenesis requires stable expression of HPV-E6 and -E7 oncogenes, these have been tumor-associated-antigens of choice for immunotherapeutic strategies. Parenterally administered therapeutic vaccines have been under active development during the past twenty years. However, none has shown enough clinical efficacies to reach commercialization. To address the role of immunization routes for inducing tumor-protection in mucosal locations (**Fig. 1**), we developed a novel orthotopic murine model for cervical cancer.[1] We compared parenteral and mucosal immunization routes for their ability to induce E7-specific cytotoxic T lymphocytes (E7-CTL) in the genital mucosa (GM), as well as protection against genital tumors (GT). Our data showed that subcutaneous (s.c.) immunization with an adjuvanted E7 polypeptide was more efficient than intranasal (i.n.) or intravaginal (ivag) routes at inducing systemic responses. The three immunization routes induced however similar numbers of E7-CTL in the GM, suggesting a better homing of these lymphocytes to the GM after i.n. and ivag immunization.[2] This is in line with the concept of a common mucosal immune system, where antigen presentation occurring in a mucosal site lead to priming of lymphocytes with a tendency to selectively home to the same or other specific mucosal sites. How far this concept can be applied to the GM has been a matter of controversy with either parenteral or different mucosal immunization routes yielding disparate results with different vaccines and/or readouts.[3,4] Superiority or efficacy of mucosal lymphocyte trafficking has however been only assessed for the induction of immune responses after infections and/or immunization or to provide protection against a pathogenic challenge. Whether this may hold true for inducing regression of mucosal tumor was to our knowledge not previously examined.

Knowing that our adjuvanted s.c. E7 vaccine provides regression of s.c. tumors through E7-CTL,[5] we anticipated that the induction of an almost identical frequency and high-avidity of E7-CTL in the GM by either of the immunization routes would predict a similar ability to induce protection in the GM. Indeed our data showed that either i.n. or s.c. immunization were able to fully prevent GT implantation. However, and surprisingly, only s.c. immunization was able to efficiently induce regression of already established E7-expressing GT, the most notable difference of s.c. immunization being the higher number of systemic and circulating E7-CTL induced, as compared with i.n. immunization. Clearly the growing tumors must influence the vaccine-induced immune response. In absence of vaccination, the growing GT induce local E7-CTL (up to 20% of the $CD8^+$ T cells), which are however counteracted by an important infiltration of $CD4^+$ $Foxp3^+$ T regulatory cells (Treg, up to 60% of the $CD4^+$ T cells).[1] Interestingly, GT regressing upon vaccination showed up to 90% E7-CTL among the infiltrating $CD8^+$ T cells, together with a decrease in Treg (less than 15% of the infiltrating $CD4^+$ T cells, unpublished data), both probably accounting for the vaccine efficacy. Although we have no clue as how vaccination led to a decrease in infiltrated Treg, our data suggest that the higher number of circulating E7-CTL present after s.c. immunization, as compared with i.n. immunization, may readily infiltrate the tumor thus explaining higher efficacy of this immunization route even in the case of a mucosal site. Lymphocyte trafficking to the GM has involved both mucosal and non-mucosal homing interactions especially upon infections.[6,7] In contrast, the growing tumors induce little innate immunity and the intratumoral vasculature appears to rather limit lymphocyte recruitment by decreasing or altering adhesion molecule expression.[8,9] It is thus the interplay between vaccination and the tumor that may lead to such an efficient

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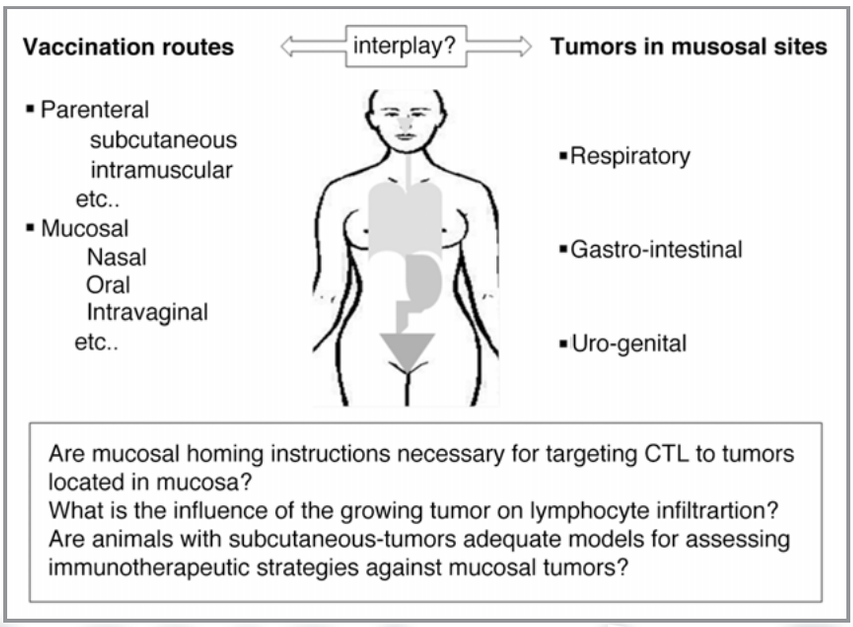

Figure 1. Vaccination routes and mucosal tumor regression.

CTL infiltration in the regressing tumor. Interestingly, systemic administration of CpG-oligonucleotides (which are used in our case as adjuvant to the E7 vaccine) was found to induce ICAM-1 and VCAM- 1 on intratumoral vessels, thus enabling strong T cell infiltrations in a pancreatic islet tumor murine model.[10] Whether this also occurs in our setting when the E7-vaccine is administered by the s.c. route, but not the i.n. route, deserve further investigation. This interplay between tumor location and vaccination routes is probably even more complex, as tumors implanted s.c. did regress after both i.n. and s.c. immunization.[2] To gain additional insights, we are examining how the same vaccine may induce regression of tumors located in another, nearby, mucosal site i.e., the bladder. Interestingly, our preliminary data suggest that homing to the bladder follow different rules than homing to the GM, with both s.c. and ivag immunization routes, but not i.n., leading to similar frequencies of E7-CTL in bladder. More surprisingly however, ivag immunization was more efficient to induce regression of tumors established in the bladder, despite lower numbers of circulating E7-CTL, a situation which is thus different from that observed for tumors located in the GM. A novel approach may be to combine vaccination with immunostimulation at the tumor location, our recent findings suggest that this not only increases locally the frequency of the vaccine-specific CTL, but also leads to a better tumor regression (unpublished data). Altogether, our results demonstrate the need to use animal tumor models that closely mimic the real situation, as well as to evaluate different immunization routes in order to optimize immunotherapeutic strategies against cancer located at different mucosal sites.

Author's View: Decrausaz L, Domingos-Pereira S, Duc M, Bobst M, Romero P, Schiller J, Parenteral but not mucosal immunization is able to induce regression of human papillomavirus associated genital tumors. Int J Cancer 2011; 129:762-72; PMID:21384340; http://dx.doi.org/10.1002/ijc.25973

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