

Nanobodies as molecular probes for targeting of dendritic cells

Dendritic cells are professional antigen presenting cells that play an important role in the control of immunity. The availability of “vehicles” to target these receptors *in vivo* is also a key issue in the challenge to manipulate the effector functions of DCs *in vivo*. Nanobodies are single-domain antigen-binding fragments derived from naturally occurring heavy-chain antibodies in *Camelidae*, constituting exquisite targeting moieties for therapeutic and diagnostic applications. In the present study, we have evaluated Nanobodies as molecular probes to target dendritic cells. Immunisation of llamas with bone marrow-derived dendritic cells resulted in the retrieval of a panel of anti-DC Nanobodies after whole cell panning. The generated anti-DC Nanobodies displayed distinct binding profiles on *in vitro* and *ex vivo* cell populations defining at least two groups of cellular specificity i.e. DC-specific and myeloid cell-specific. To evaluate the application of anti-DC Nanobodies as targeting moieties for delivery of antigens to DCs, we have genetically fused a model Ag, with selected Nbs. Nanobody-mediated targeting to DCs could enhance antigen presentation of the truncated OVA by the DCs, but so far the observed antigen presentation was restricted to the MHC class I pathway. In order to explore the role of anti-DC Nanobodies as imaging agents, the biodistribution of radionuclide labelled Nanobodies was evaluated by single photon emission computed tomography after intravenous injection in naïve mice. The observed *in vivo* biodistribution for the selected Nanobodies with different cellular specificities nicely reflects the main *in vivo* locations of the cells that have been determined *in vitro* to be recognized by the Nanobodies. We verified the observed biodistributions of the Nanobodies by grafting the antigen recognition loops upon a universal scaffold and re-evaluated their biodistributions. These experiments have indicated that the observed biodistribution of these Nanobodies is determined by their antigen-binding loops. In addition to the ‘classical’ Nanobodies, we exploited for the first time a novel class of Nanobodies, in essence Nanobodies of the family IV which expand the known repertoire.