Salmonella- based DNA vaccine

1. Aim of the project

This project involves the use of genetically modified *Salmonella typhimurium* that has the capacity, like the non genetically modified counterparts, to enter **A**ntigen-**P**resenting **C**ells (APCs, for instance macrofages or dendritic cells) upon intake through the enteric route. Taking advantage of this feature, *S. typhimurium* will be transfected with a vector giving the bacteria the ability to function as an oral carrier for genetic immunization (e.g. DNA-vaccine). It involves APCs capacity to transcript the insert that has been cloned into the vector of the GM bacteria and to cut the product of this insert in small peptides (antigens). Then, these antigens are presented on the surface of the APCs via Major Histocompatibility Complex (MHC) molecules (in a mouse model) or HLA molecules (in humans). This project investigates whether an animal (e.g. mouse), which has taken up the GM bacteria, will develop an immune response against antigens that are specific to the insert product thanks to the *Salmonella*-mediated transfer of genes to eukaryotic (APC) cells.
Finally, this oral carrier for genetic immunization could have medical applications by presenting antigens via HLA (Human Leukocyte Antigens).

2. Vector/ plasmid :

Mammalian expression vector that is well characterised and commercially available (type pcDNA 3.1 zeo, Invitrogen) with:
- Commonly used selectable marker gene(s) of mammalian or bacterial origin (e.g. beta-lactamase coding region, zeocin resistance gene). These gene products confer resistance to antibiotics that are not used as a last resort in the medicinal or veterinary field.
- Commonly used sequences of viral origin to promote expression of cloned inserts and selectable markers (e.g. human cytomegalovirus (CMV) immediate-early enhancer/promoter region, simian virus 40 (SV40) enhancer and 'early' promoter)
- Commonly used sequences of viral origin to provide transient, episomal replication in cells expressing SV40 large T antigen such as COS-1 or COS7 cells.

Such vectors can be amplified in a prokaryotic system, but the inserts will not be expressed in a prokaryotic environment. Likewise, the GM *S. typhimurium* discussed in this example will not express the cloned insert. Expression of the insert will only occur in a mammalian host, for instance after *Salmonella*-mediated gene transfer into APCs.

3. Insert :

The insert is a wild type coding sequence for murine heparanase. To allow a high level of expression, enhance-promoter sequences of the 'immediate early' gene of human cytomegalovirus (CMV) are used. Heparanase is involved in the degradation of heparan sulfate proteoglycans, which are key components of the extracellular matrix. Increased levels of heparanase activity are associated with several tumor types and tumor cells transfected with heparanase cDNA become more invasive and acquire a highly metastatic phenotype.

4. Localisation of inserted genetic material:

Episomal (transient expression).

5. Receptor organism:

The receptor organism is a genetically modified bacterium of class risk 1, more precisely an attenuated *aroA* mutant of *Salmonella typhimurium* that is deficient for replication. This strain has lost all virulent traits due to a deletion that prevents the synthesis of essential metabolites. These metabolites are neither present in mammalian hosts resulting in the inability of these bacteria to survive upon oral intake. Restoration of the wild-type phenotype with virulent traits is unlikely to happen since the genetic modification is a deletion mutation.

6. Biological hazards and/or considerations related to the manipulation of the resulting GMM

- Manipulations with the GMM are performed on a laboratory scale and involve cloning techniques, centrifugation, pippetting, etc. To test the functionality of the resulting GMM as an oral carrier for DNA-vaccin, small laboratory animals are treated with the GMM (oral intake of GM *S. typhimurium*).
- The GMM is derived from a non-virulent strain of *S. typhimurium*. No *S. typhimurium* can be found in faeces of infected animals, transmission by faecal-oral route is unlikely.
- The GMM is unable to express the insert that has been cloned in the mammalian expression vector.
- Beside cells that are naturally infected by *S. typhimurium* (like APCs) it is very unlikely that other eukaryotic cells of the host would take up the DNA insert and express the coding sequences. Moreover, by choosing a mammalian vector that allows transient and episomal expression, the insert will be expressed for a limited period of time in the APCs.
- Given the worst case that the manipulator would take in a large amount of the GM *S. typhimurium*, the bacteria will normally follow the enteric route and the processing by APCs will possibly induce an immune response against murine heparanase epitopes.

7. Class of risk of resulting GM organism.

The GM *S. typhimurium* bacteria can be assigned to class of risk 1.

8. Class of risk of the activity

The activity involves standardized manipulations on laboratory scale and can be assigned to class of risk 1 (cfr Annex III of Directive 98/81/EC).

9. Recommended containment measures

Biosafety level 1.
An animal facility of biosafety level 1 is sufficient for the containment of laboratory animals treated with GM *S. typhymurium*.

10. References and further reading

Paglia P, Medina E, Arioli I, Guzman CA, Colombo MP. (1998). Gene transfer in dendritic cells, induced by oral DNA vaccination with *Salmonella typhimurium*, results in protective immunity against a murine fibrosarcoma.Blood. 92(9), 3172-6.

Reiland J, Sanderson RD, Waguespack M, Barker SA, Long R, Carson DD, Marchetti D. (2004). Heparanase Degrades Syndecan-1 and Perlecan Heparan Sulfate : Functional Implications for Tumor Cell Invasion. J Cell Biochem 93 (2), 215-23