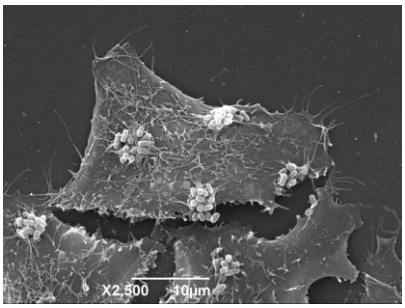
An engineered bacteria to deliver intracellular single domain antibodies into human cells.

Scientists at the National Center for Biotechnology, CNB-CSIC, have developed non-invasive *Escherichia coli* bacteria carrying functional molecular syringes assembled by a Type III protein secretion system (T3SS) that are able to secrete and translocate to the cytoplasm of human cells single domain antibodies (sdAb) fragments with full capacity to bind to their cognate antigens. Moreover, they have shown their functionality by the formation of antigen-sdAb complexes in the cytoplasm of infected cells. The use of live bacteria has a great potential for delivery of therapeutic proteins in vivo. Industrial partners interested in a patent license are being sought.

An offer for Patent license

Direct transfer of antibody fragments from *E. coli* into target cells has an enormous biotechnological and therapeutic potential.

The smallest Ab fragments (12~15 KDa) are the so-called single domain antibodies (sdAbs), which are composed of a single variable (V) immunoglobulin (Ig) domain. The sdAbs from camelid are heavy-chain-only Igs known as VHH domains or nanobodies. Nanobodies appeared specially suited for this application given their potential as enzyme inhibitors, monomeric nature, stability and size (2-3 nm) that fit in the protein channels of bacteria syringes. The proof of concept has been done with intestinal pathogenic *E. coli* strains that use a T3SS to inject specific bacterial proteins referred to as "effectors", into mammalian cells. An attenuated *E. coli* strain lacking all of its effectors or commensal *E. coli* strains endowed with a functional T3SS are being engineered.



Scanning electron micrograph of human HeLa cells infected in vitro with attenuated EPEC bacteria carrying a functional T3SS injecting sdAbs into the cytoplasm of the human cell.

Main innovations and advantages

- Non-invasive *E. coli* cells carrying a Type III protein secretion system remain extracellular and can inject specifically the desired single domain antibodies.
- The levels of intracellular sdAbs (10^5-10^6 molecules per cell) are appropriated to modulate the activity of regulatory and cell-signaling proteins.
- Injection of sdAbs does not require bacterial invasion or the transfer of genetic material, differing from other approaches that need the transfer of the protein-encoding gene by viral infection or transfection.

Patent Status

Patent pending in USA.

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