A recombinant *Bacillus subtilis* vaccine to promote MHC class I-dependent antigen presentation – preliminary results.

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Background

The ability to generate antigen-specific CD8+ T cells is crucial in mediating protection of eukaryotic cells against intracellular bacteria (Seder and Hill, 2000). T cell response depends on antigen presentation on the surface of specialized antigen presenting cells (APCs), such as dendritic cells (DCs) or macrophages. Delivery of antigens directly to the cytosol of the APCs is now being widely examined. Some live vaccine carriers, such as intracellular bacteria can survive inside the APCs, thereby delivering the antigens directly to the cytosol and their presentation in association with MHC class I molecules (Detmer and Glenting, 2006). However, pathogenicity of such vaccine vectors has prompted the search for other carriers based on non-pathogenic bacteria.

Objective

The aim of this study was to examine whether *Bacillus subtilis* strain expressing *Listeria monocytogenes* protein listeriołysin O (LLO) ałong with different variants of a modeł antigen ovałbumin (OVA), is abłe to induce presentation of OVA in the context of MHC class I of JAWS II DCs.

Methods

Strains producing recombinant antigens were obtained by standard methods of molecular cloning, including creation of fusion genes by splicing by overlap extension method, their cloning in expressive and integrative vector, and incorporation of exogenous genetic material by chemical transformation. The OVA gene or its partial sequences were introduced into the pUR10 plasmid carrying the *hly* gene coding listeriolysin O of *Listeria monocytogenes* (**Fig. 1)**. Recombinant proteins of *B. subtilis* strains were visualised by SDS-PAGE (**Fig 2**). Intracellular proliferation of the mutant *B. subtilis* strains was evaluated in the gentamicin protection assay. Level of OVA presentation in the complexes with MHC class I molecules was examined *in vitro* by using specific 25D-1.16 antibody (Porgador et al., 1997) recognizing the conjugate and determined by flow cytometry.

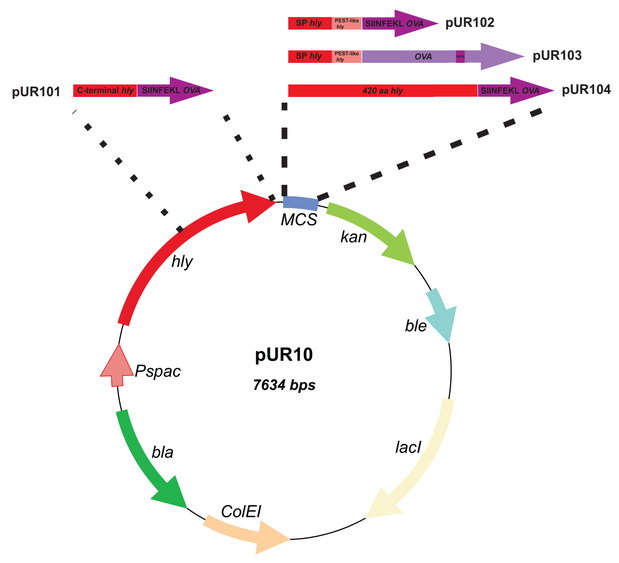


Fig. 1 Construction of the pUR10-based plasmids carrying fragments or whole sequence of the OVA antigen. Vectors pUR101 - pUR104 were achieved respectively by cloning: **a)** OVA epitope (SIINFEKL) sequence fused to C-terminal sequence of LLO, **b)** fusion sequence LLO-OVA (N-terminal sequence of LLO coding ribosome binding site (RBS), signal peptide (SP) and PEST-like sequence fused to the sequence of SIINFEKL), **c)** fusion sequence LLO-OVA (N-terminal sequence of LLO coding RBS, SP and PEST-like sequence fused to the complete sequence of OVA), **d)** partial LLO sequence coding first 420 aa fused to SIINFEKL sequence). Truncated form of LLO lacking 84 C-terminal amino acids (domain 4 of the protein) was used as it retains its activity and is essential for IFN-γ-inducing activity, what plays an important role especially in inducing protective immunity.

Results

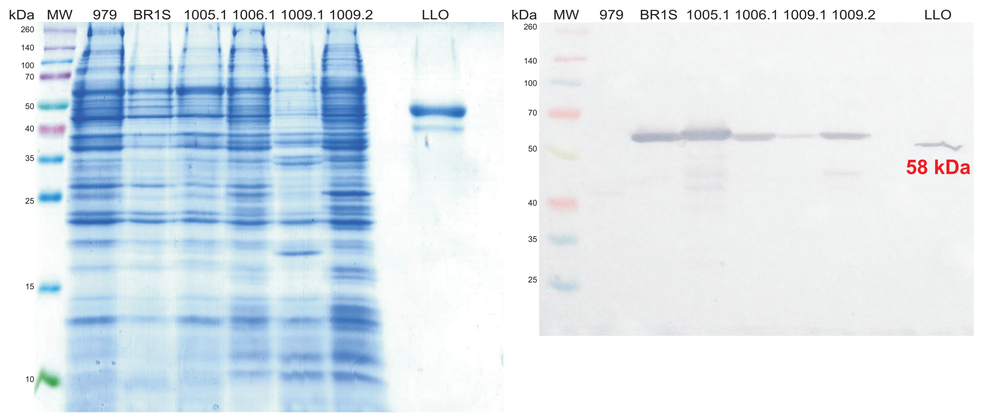
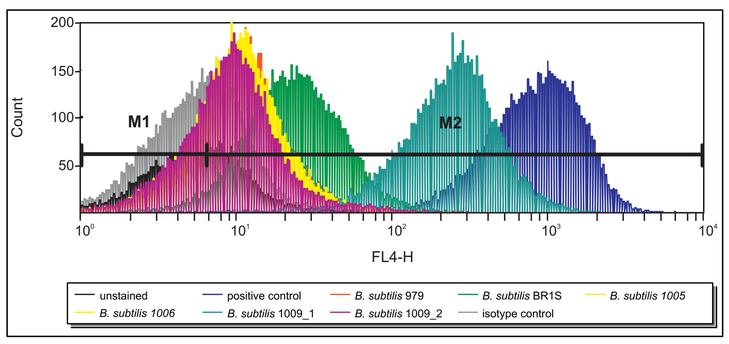


Fig. 2 The analysis of immunogenic peptide secretion by LLO-OVA *Bacillus subtilis* **strains.** Supernatant proteins were isolated from the culture supernatants of IPTG-inducted strains by TCA / ethanol precipitation, and subsequently subjected to SDS-PAGE gels. For Western-blotting, gels were blotted onto PVDF membrane, which, after blocking, was incubated with primary rabbit anti-LLO antibody and secondary goat anti-rabbit antibody conjugated with alkaline phosphatase.



Sample	Gate	# of Events	% of gated cells	Median	Geometric Mean	CV	Peak Value	Peak Channel
unstained	M1	6186	58,42	3,65	3,28	42,54	286,00	1,00
	M2	4403	41,58	9,65	10,95	83,07	228,00	6,73
isotype control	M1	11745	56,10	3,79	3,39	41,86	486,00	1,00
	M2	9192	43,90	10,00	11,28	422,46	443,00	6,73
positive control	M1	78	0,37	3,22	3,02	44,23	5,00	2,74
	M2	20958	99,63	835,36	767,99	67,07	447,00	1000,00
non-infected	M1	9337	44,33	3,92	3,56	39,80	414,00	6,04
	M2	11724	55,67	10,75	11,73	94,70	502,00	8,98
B. subtilis 979	M1	4782	21,99	4,53	4,04	34,51	313,00	6,49
	M2	16969	78,01	12,41	13,63	114,10	581,00	11,56
B. subtilis BR1S	M1	887	4,11	4,87	4,28	31,75	68,00	6,04
	M2	20692	95,89	24,58	25,14	91,93	465,00	22,88
B. subtilis 1005_1	M1	5759	26,78	4,53	4,04	34,38	384,00	6,49
	M2	15749	73,22	11,97	13,26	127,39	566,00	9,65
B. subtilis 1006_1	M1	4756	21,92	4,53	4,09	33,79	316,00	6,49
	M2	16940	78,08	12,41	13,77	113,85	598,00	9,31
B. subtilis 1009_1	M1	70	0,32	3,34	2,98	50,60	7,00	1,00
	M2	21547	99,68	245,82	231,81	74,14	567,00	245,82
B. subtilis 1009_2	M1	6328	29,50	4,53	3,96	35,27	397,00	6,49
	M2	15126	70,50	11,55	13,23	202,33	566,00	9,65

Fig. 3 Differences in OVA epitope (SIINFEKL) presentation by JAWS II dendritic cells (DCs) infected with different strains of *B. subtilis* **producing LLO-OVA fusion proteins.** Presentation of immunogenic peptide was evaluated by flow cytometry after staining DCs infected by *B. subtilis* with BD Biosciences 25D-1.16 antibody capable of recognizing oktapeptide SIINFEKL in a complex of MHC class I molecules (H2-K^b). DCs pulsed with a wild-type strain *B. subtilis* 979 (non-haemolytic) and BR1S (haemolytic) strains were included as negative controls, while DCs pulsed with 10 ng/ml SIINFEKL peptide were used as a positive control of peptide presentation. Data were aquired by FACSCalibur cytometer and processed using FCS Express 4 Flow programme (De Novo Software).

Conclusions

Preliminary cytometric evidence revealed that *B. subtilis* strain carrying LLO-OVA epitope fusion protein (1009_1) is capable of efficient delivery of the OVA antigen to the cytosol of APCs, resulting in presentation of the antigen in the context of MHC I, and thus enabling induction of T cell response.

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References

Albaghdadi H., Robinson N., Finlay B., Krishnan L., Sad S. 2009. Selectively reduced intracellular proliferation of *Salmonella enterica* serovar Typhimurium within APCs limits antigen presentation and development of a rapid CD8 T cell response. *J Imm* 183: 3778-3787.

Detmer A, Glenting J. 2006. Live bacterial vaccines – a review and identification of potential hazards. *Microb Cell Fact* 5: 1-12.

Porgador A., Yewdell J. W., Deng Y, Bennink J. R., Germain R. N. 1997. Localization, quantitation, and in situ detection of specific peptide-MHC class I complexes using a monoclonal antibody. *Immunity* 6: 715-726.

Seder R. A., Hill A. V. 2000. Vaccines against intracellular infections requiring cellular immunity. *Nature* 406: 793-798.