Pyrococcus furiosus thioredoxin as a platform to express extracellular loops of Treponema pallidum outer membrane proteins to assess antigenicity and isolate antigen-specific B-cells: a new structural approach for syphilis vaccine development

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Background

The incidence of syphilis continues to increase in the US and globally. Development of an effective syphilis vaccine is a critical component of strategies to curtail this surging public health threat. Syphilitic infection in rabbits has demonstrated that it is possible to elicit a protective response against Treponema pallidum. Immune rabbit serum (IRS) enhances oncoprophagocytosis of intact spirochetes by macrophages, indicating the presence of protective antibodies targeting surface epitopes. Our hypothesis is that antibodies directed against extracellular loops (ECLs) of outer membrane proteins (OMPs) are responsible for the opsonic activity observed in IRS. The Pyrococcus furiosus thioredoxin (PfTrx) was used as a scaffold to express ECLs (PfTrx-ECL), which was then used to assess ECL reactivity with syphilitic sera and isolate ECL-specific B-cells.

PTTrx construct design and cloning

PTTrx was engineered to express BamA-ECL4 (PTTrxBamA/ECL4), a highly immunogenic and opsonic loop previously characterized by our group. N-terminal His6- and C-terminal Avi-tags were added to PTTrxBamA/ECL4 for Ni-NTA purification and in vivo biotinylation, respectively (Fig. 1).

PTTrx BamA-ECL4 expression and purification

PTTrxBamA/ECL4 was then cloned into pET28a expression vector and transformed into E. coli BL21 containing the biotin-protein ligase BirA, which allows for in vivo biotinylation. Recombinant proteins were purified from the supernatant in three-steps: (i) heat purification, (ii) Ni-NTA affinity column and (iii) size exclusion chromatography (Fig. 2).

Screening for ECL antigenicity

The antigenicity of PTTrxBamA/ECL4 with IRS (Nichols strain) and human secondary syphilitic sera (HSS) was assessed by immunoblotting. IRS and HSS reacted with PTTrxBamA/ECL4, cross-reactivity against PTTrx was not detected. (Fig 3).

ECL accessibility for antibody recognition

A pull-down assay was done evaluate the ability of PTTrxBamA/ECL4 to present ECLs in an antibody accessible form, PTTrxBamA/ECL4 (or PTTrx alone) was incubated with IRS or NRS and protein G agarose beads. PTTrxBamA/ECL4 was eluted from Nichols IRS but not NRS. Importantly, PTTrx alone was not pulled down with either IRS or NRS (Fig. 4).

BamA/ECL4-specific B-cells

PTTrxBamA/ECL4 then was used to identify Ag-specific B-cells from PBMCs of an immune rabbit by flow cytometry. Rabbit B-cells of the IgG isotype were identified as shown in Fig. 5. 16.6% of the PTTrxNsg IgG+ cells were PTTrxBamA/ECL4-specific.

Conclusions

- The PTTrx ECL system described represents a major step forward to assess antigenicity of ECLs and dissect the opsonic activity of IRS.
- PTTrx present ECLs in an accessible form for antibody recognition as confirmed by the pull-down assay.
- PTTrx-ECL constructs can be used to identify ECL-specific B-cells in rabbit PBMCs and generate ECL-specific mAbs.
- This system will help to identify highly immunogenic and opsonic ECLs as potential syphilis vaccine candidates.

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References