



Interferon genes regulated by pertussis toxin: potential for an in vitro pertussis vaccine safety assay?

Introduction

Pertussis vaccines have proven very effective in decreasing the incidence of whooping cough. Since these vaccines are routinely administered to healthy infants and contain inactivated bacterial components, tests to ensure safety of these vaccines are of critical importance. The histamine sensitization test (HIST) is currently the standard assay to test for the absence of active pertussis toxin (PTx). Being a lethal animal test that is difficult to standardize, replacement of the HIST is a priority. Moreover, the exact mechanism of the test is undefined, nor is it clear whether the assumed underlying mechanism, i.e. PTx-mediated ADP-ribosylation of G proteins, is the only relevant effect of PTx.

An alternative way to develop an assay to replace animal testing

Aim of our project is to develop a novel mechanism-based cell assay to detect residual PTx activity in vaccines.

- The assay should reflect clinically relevant PTx effects.
- The assay should be specific, sensitive and robust.
- Assay development is based on the 'assuring safety without animal testing' (ASAT) principle (ASAT, reversing the paradigm in toxicity testing, B. Sangster, www.alttox.org).

Clinical effects of PTx and underlying mechanisms

| Clinical effect | Effect of PTx |
|--------------------------------|--|
| Hyperinsulinemia, Hypoglycemia | Increases insulin secretion of pancreatic β cells |
| Pulmonary hypertension | Induces leukocytosis, inhibits extravasation of immune cells |
| Pneumonia | Induces leukocytosis, increases vascular permeability |
| Systemic hypotension | Affects contractile apparatus of smooth muscle cells |
| Neurological problems | Increases vascular permeability and possibly direct effects |

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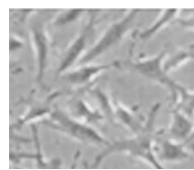
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Involved cells

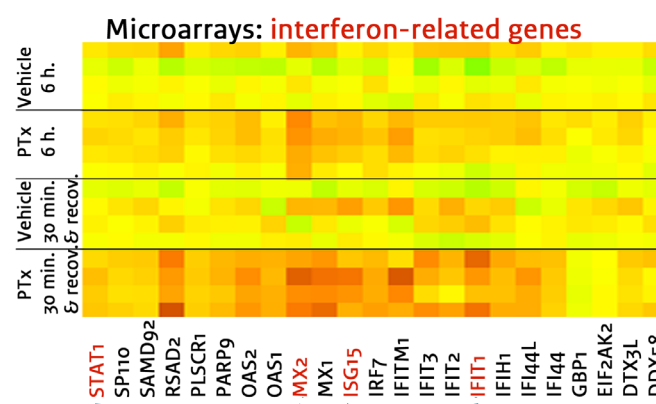
- Pancreatic β cells
- Smooth muscle cells
- Barrier cells (endothelial and epithelial)
- Immune cells (neutrophils, macrophages, dendritic cells and T cells)

Procedure and results

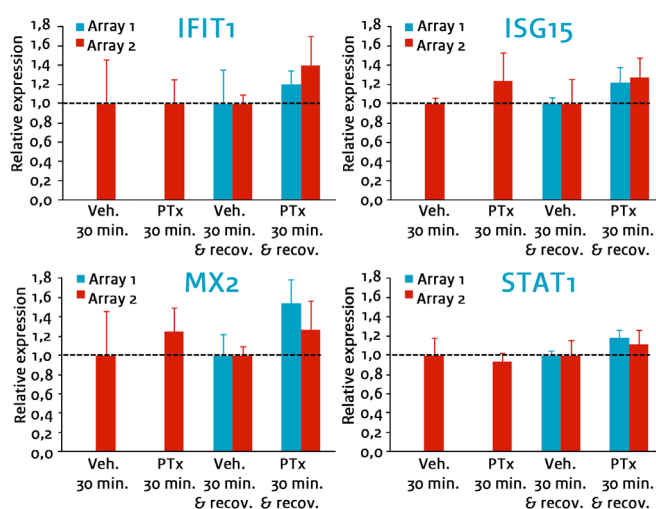


Cell line: **EA.Hy926**
Hybrid of Human umbilical vein endothelial cells & A549 lung epithelial cell line
Relevance: Human & cell types involved in clinical effects of PTx

Incubations: Pertussis toxin (PTx): 90/518, NIBSC, 250 ng/ml i.e. 26.25 IU/ml, various durations



Q-RT-PCR to validate microarray results



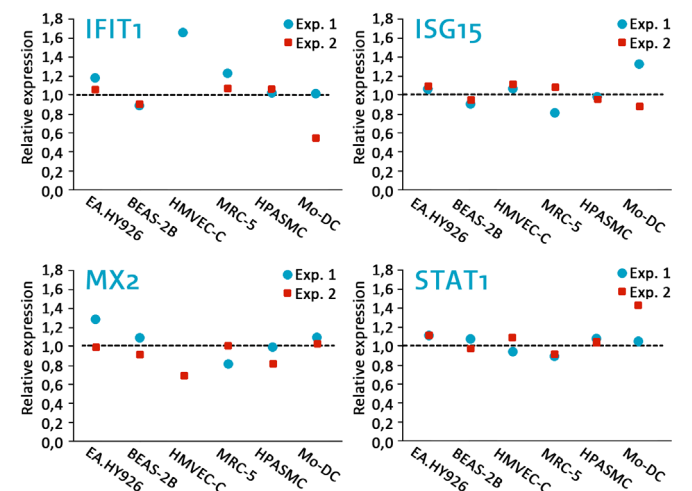
Confirming results in different cell types

In a next step, we examined the effect of PTx on the 4 selected interferon-related genes in 5 other relevant cell types:

| Name of cell line/type | Description |
|------------------------|--|
| BEAS-2B | Human pulmonary epithelial cell line |
| HMVEC-C | Primary, human microvascular cardiac endothelial cells |
| MRC-5 | Human fetal pulmonary fibroblast cell line |
| HPASMC | Primary, human pulmonary artery smooth muscle cells |
| Mo-DC | Human monocyte-derived dendritic cells |

Cells were incubated for 2 hours with 250 IU/ml PTx (JN1H-5, NIBSC), i.e. 250 ng/ml, or vehicle.

After incubation, RNA was isolated and converted to cDNA before performing quantitative-realtime-PCR for the 4 selected interferon-related genes. Experiments were performed twice, quadruplicates within each experiment were pooled.



Conclusion

Although the initial micro-array data indicate that PTx induces interferon-related genes in EA.Hy926 cells, subsequent experiments in this cell line and 5 additional relevant cell lines indicate that the PTx-induced expression of these genes is insufficient for these genes to be useful as marker-genes for PTx.

Future plans

Microarray experiments with the additional 5 mentioned cell lines/types will be performed to find other genes that are specifically and strongly affected by PTx and that can be used as reporter genes to detect the presence of PTx.