The live attenuated *B. pertussis* vaccine strain BPZE1 subverts the immune functions of RSV-infected human dendritic cells

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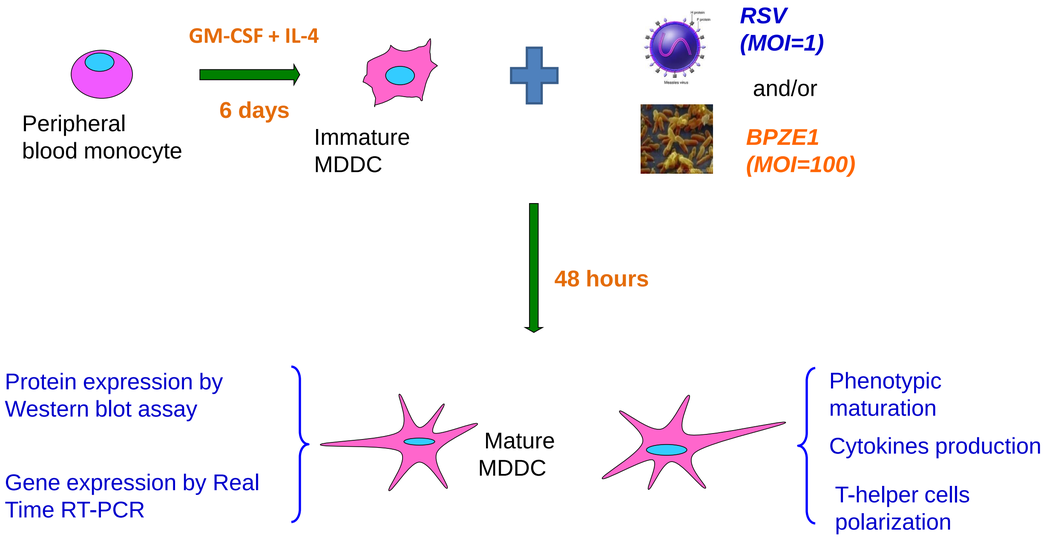
INTRODUCTION AND AIMS

The respiratory syncytial virus (RSV) is the most common cause of childhood acute lower respiratory tract infection and a major cause of admission to hospital. In recent years many advances have been made in the knowledge of the immunologic and inflammatory factors that contribute to RSV-disease. The emerging pathogenic view implies that infection may induce a cytokine storm leading to a final outcome of immune pathology and tissue damage.

Recently, a live attenuated *Bordetella pertussis* vaccine strain, BPZE1, in which three virulence factors (the pertussis toxin, the dermonecrotic toxin and the tracheal cytotoxin) have been eliminated or detoxified, was developed and entered a phase I safety trial. Remarkably, it has been shown that immunization with BPZE1 protects mice challenged with a highly pathogenic influenza virus, likely involving reduction of cytokine-mediated inflammation in the lungs and priming of T-helper cells. On the basis of these observations and to support the potential application of BPZE1 as adjuvant in a future RSV vaccine, we have assessed the immunomodulatory properties of BPZE1 in a model of human monocyte-derived dendritic cells (MDDC) infected with RSV.

EXPERIMENTAL DESIGN



RESULTS

Human MDDC double infected with BPZE1 and RSV acquire a mature phenotype and express both IL-10 and IL-12p70

MDDC were infected with RSV, BPZE1 or their combination. RSV infection induced semi–mature MDDC, expressing enhanced levels of CD80, HLA-DR and CD38 but not CD83. Full blown maturation was induced by BPZE1 or by RSV and BPZE1 co-treatment (Figure 1A). RSV-infected cells did not release IL-10, while BPZE1 induced high levels of this regulatory cytokine; barely detectable levels of Th1-driving IL-12p70 were expressed by either RSV- or BPZE1-MDDC. Remarkably, in double infected cells, an increased release of IL-12p70 was observed (Figure 1B).

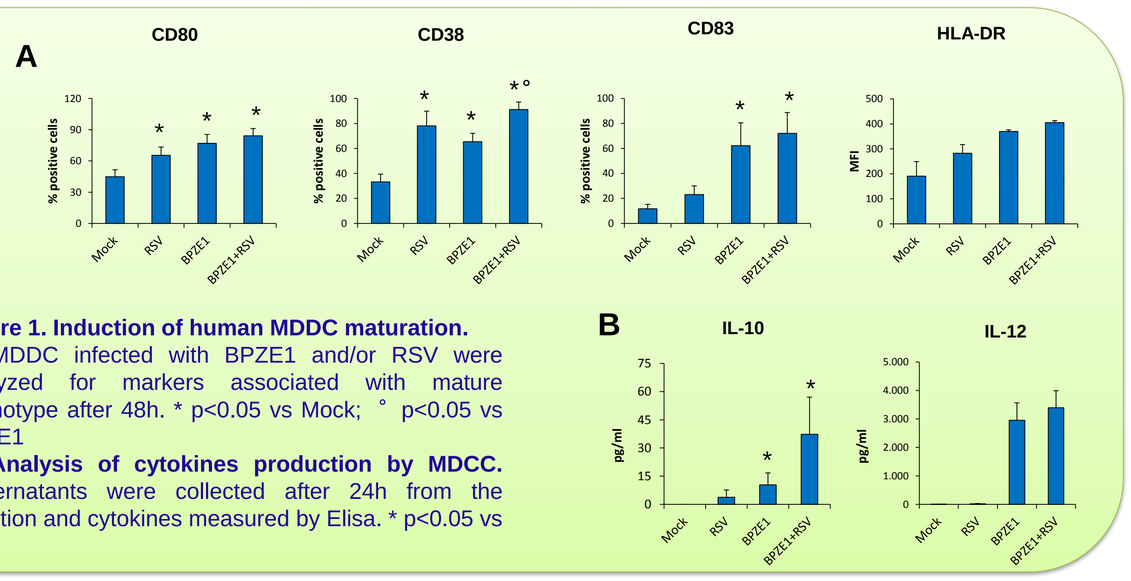


Figure 1. Induction of human MDDC maturation.
A. MDDC infected with BPZE1 and/or RSV were analyzed for markers associated with mature phenotype after 48h. * p<0.05 vs Mock; ° p<0.05 vs BPZE1
B. Analysis of cytokines production by MDCC. Supernatants were collected after 24h from the infection and cytokines measured by Elisa. * p<0.05 vs RSV

BPZE1 shifts the polarization ability of RSV-infected MDDC towards a mixed Th1/Th17 profile.

MDDC treated with BPZE1 induced the production of IFN-γ and IL-17 by co-coltured T cells, indicating a mixed Th1/Th17 polarization; MDDC stimulated with RSV induced production of IFN-γ and IL-5/IL-4, testifying a mixed Th1/Th2 polarization; double infected MDDC switched the polarization of T lymphocytes towards a mixed Th1/Th17 profile, as compared to RSV alone, with a marked reduction of Th2 cytokines.

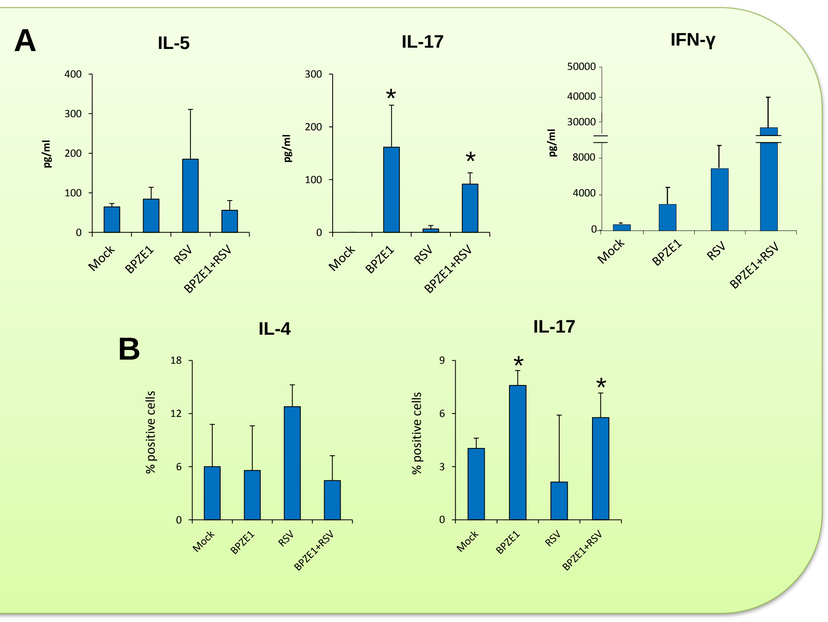


Figure 2. T Lymphocyte polarization.
A. MDDCs were treated with RSV and/or BPZE1 for 48 h and then co-cultured with CD3 cells. On day 12, supernatants were harvested for cytokine measurement. Values are indicated as means±SE of four experiments *p<0.05 vs RSV;
B. MDDC were co-cultured with CD3 cells. On day 12 cells were activated with PMA and ionomycin in the presence of brefeldin A and cytokine measured by intracellular staining. Values are indicated as means±SE of four experiments p<0.05 vs RSV;

RSV and BPZE1 induce different intracellular signalling pathways in MDDC

RSV induced the phosphorylation of Stat1, belonging to IFNs-mediated Jak/Stat pathway. BPZE1 induced the phosphorylation of key proteins belonging to the MAPK family and involved in activation of NF-κB. In double infected cells both pathways were activated.

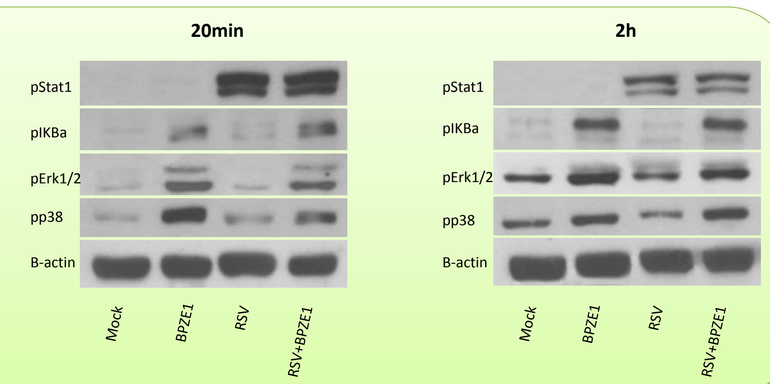


Figure 3. Analysis of MyD88 dependent and independent pathways induction.
MDDC were either treated with BPZE1 and/or RSV. Phosphorylation of Stat1, IκB-α, ERK1/2, p38 was determined at the indicated time-points by Western blot.

RSV and BPZE1 complement each other to induce the expression of a wider array of cytokines and chemokines

BPZE1 induced the pro-inflammatory cytokines IL-6 and the p40 subunit of IL12p70, both known to be regulated by the MAPK pathway, while RSV induced the expression of IFN-β, IFN-regulated chemokine Rantes and the p35 subunit of IL-12p70. In double infections expression of all these genes was induced. Expression of both IL-12p40 and IL-12p35 subunits was in agreement with enhanced IL-12p70 production measured by ELISA.

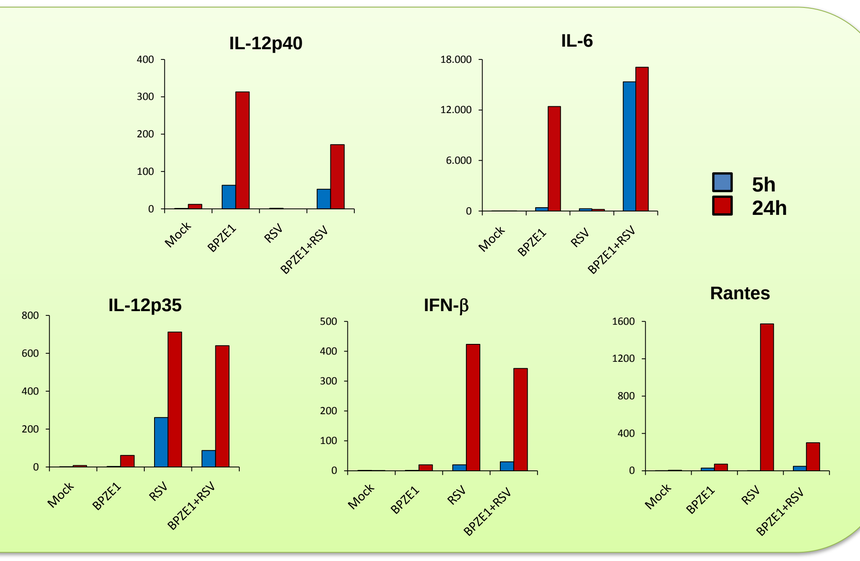


Figure 4. Gene expression induced by BPZE1 and RSV infection.
MDDC were either treated with BPZE1 and/or RSV and the mRNA extracted at indicated time points used to measure the expression of genes by quantitative RT-PCR.

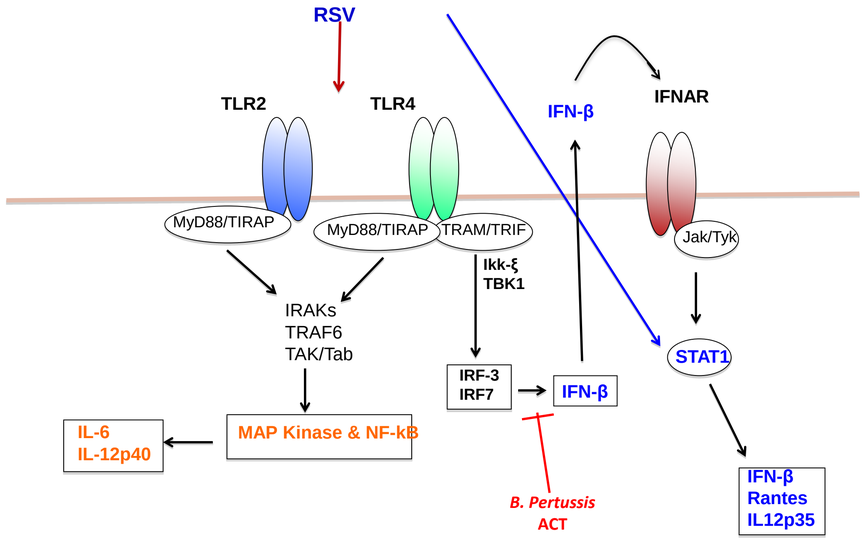
CONCLUSIONS

➢ RSV-infected MDDC have a semi-mature phenotype and do not release cytokines. RSV infection triggers the phosphorylation of STAT1 in MDDC and the expression of type-I IFN and IFN-dependent genes

➢ BPZE1 subverts MDDC response to RSV triggering the activation of the MAPK pathway. As a results double-infected cells reach a full maturation state, release the Th1-driving cytokine IL-12p70, and shift polarization of Th cells from a Th1/Th2 to a Th1/Th17 response.

➢ BPZE1 is able to re-direct the immune response to RSV. These results support a potential application of BPZE1 as adjuvant in a future RSV vaccine.

Intracellular pathways induced by RSV - BPZE1 in dendritic cells



REFERENCES

González P., Bueno S., Carreño L., Riedeland C., Kalergis A.. **Respiratory syncytial virus infection and immunity.** *REVIEWS IN MEDICAL VIROLOGY*. Aug. 2011.

Li R., Lim A., Phoon M., Narasaraju T., Ng J., Poh W., Sim K., Chow V., Locht C., and Alonso S. **Attenuated Bordetella *pertussis* Protects against Highly Pathogenic Influenza A Viruses by Dampening the Cytokine Storm** *JOURNAL OF VIROLOGY*, Apr. 2010,

Spensieri F., Fedele G., Fazio G., Nasso M., Stefanelli P., Mastrantonio P., Ausiello C., **Bordetella pertussis inhibition of interleukin-12 (IL-12) p70 in human monocyte-derived dendritic cells blocks IL-12 p35 through adenylate cyclase toxin-dependent cyclic AMP induction.** *INFECTION AND IMMUNITY,* May 2006.

Fedele G., Bianco M., Debrie A., Locht C. and Ausiello C. M. **Attenuated Bordetella pertussis Vaccine Candidate BPZE1 Promotes Human Dendritic Cell CCL21-Induced Migration and Drives a Th1/Th17 Response** *JOURNAL OF IMMUNOLOGY,* Mar. 2011.

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