Inhibition of phosphatidylinositol-3-kinase- δ improves tissue destruction in pemphigoid diseases by impairing neutrophil function

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Introduction

Phosphatidylinositol-3-kinase (PI3K) δ -dependent pathways are essential for immune cell activation. Targeting PI3K- δ has beneficial effect in several preclinical models of inflammatory disorders. PI3K- δ contributes to T- and B-cell development, survival, and antigen recognition as well as various neutrophil functions. Here, we investigated the effect of INCB050465, a novel PI3K- δ inhibitor, on disease-driving pathways in the pemphigoid diseases (PDs) namely bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) as autoantibody-induced and neutrophil-dependent diseases.

Methods

In vitro study

- ROS release assay:** It is a luminol-amplified chemiluminescence technique which models the immune complex (IC)-induced PMN activation in PDs. Upon stimulation of PMNs using ICs, consisting of recombinant human-NC16A antigen/murine COL7 antigen and BP patient IgG/anti-murine IgG, they generate ROS which lead to oxidation of luminol and emission of blue luminescence that is detectable by a luminometer.

- Cell-free ROS release assay:** This system comprises glucose/glucose oxidase (G/GOX), catalase (CAT), and myeloperoxidase (MPO) that enables regulated generation of hypochlorous acid (HClO) and H₂O₂ that simulates the oxidative burst of neutrophils.

- Cryosection assay:** An approach that mimics PMN-induced tissue damage (split formation) in PDs.

- Cytotoxicity assay:** a flow cytometry-based assay using Annexin V (AV) and propidium iodide (PI) for detection of viable, early and late apoptotic cells.

In vivo study

- Immunization-induced EBA:** B6.s mice were immunized with 120 μ g/head vWFA2 protein (immunodominant domain of murine type VII collagen) and Titermax as an adjuvant. When percentage of affected body surface area reached >2, mice were randomly allocated to treatment either with drug or vehicle (5% (v/v) N,N-Dimethylacetamide (DMA) in methylcellulose (0.5%)). Methylprednisolone (MP) was utilized as a reference treatment.

Treatment	Dose	Route	Frequency
INCB050465	0.3 mg/kgBW	p.o.	2X/day
INCB050465	3 mg/kgBW	p.o.	2X/day
Vehicle		p.o.	2X/day
MP	20 mg/kgBW	i.p.	1X/day

Results

- PI3K- δ inhibition decreases ROS generation by IC-activated PMNs.**

- ROS-inhibition of INCB050465 was not attributed to its anti-oxidant activity.**

- Split formation is hampered by PI3K- δ inhibition in a dose dependent manner.**

- INCB050465 demonstrated no drug-induced toxicity in PMNs.**

- PI3K- δ inhibition hinders disease progression in established immunization-induced EBA.**

Conclusion

- PI3K- δ inhibition led to:
- impairment of ROS generation by IC-stimulated PMNs.
  - diminution of Ab-mediated tissue damage by PMNs.
  - reduction of disease progression in established immunization-induced EBA.