SUMMARY

Patients suffering from viral respiratory diseases are often infected with several unrelated viruses, known as co-infection. However, it is unclear how viral co-infections influence disease pathogenesis. Our lab previously established a respiratory viral co-infection mouse model to better understand how viral coexistence influence pathogenicity and severity of respiratory diseases. The goal of this research was to test the hypothesis that mice infected with RV1B/PR8 and RV1B/PVM co-infections have lesser pathology than mice infected by individual viruses. Mice were inoculated with a mild respiratory virus (rhinovirus; RV1B) two days before a virus that causes severe disease (influenza A virus called PR8 or pneumonia virus of mice termed PVM). Bronchoalveolar lavage (BAL) samples were collected from lungs of mice infected with PR8 or PVM alone, or mice co-infected with RV1B and either PR8 or PVM. They were used to quantify leukocytes present in the airways of mouse lungs. In addition, lung samples were fixed in formaldehyde and embedded in paraffin. Paraffin sections were stained with Masson’s tri-chrome stain and a hematoxylin and eosin (H&E) stain to evaluate inflammation and tissue damage. The results suggest that compared to single respiratory viral infections, co-infections decreases the severity of lung pathology.

METHODS & ANALYSIS

HISTOLOGY STAINS

- All mice lung samples were collected at 6 days post infection.
- Paraffin embedded tissues were sectioned and stained with: Hematoxylin & eosin (H&E): nucleus (dark blue); cytoplasm and red blood cells (pink).
- Masson’s tri-chrome: collagen (blue); nucleus (dark brown); hyaline membrane (light purple).

LEUKOCYTE COUNT IN BRONCHOALVEOLAR LAVAGE (BAL)

- Macrophages: phagocytic innate immune cells.
- Neutrophils: inflammatory innate immune cells.
- Lymphocytes: pathogen specific adaptive immune cells.

H&E

Tissue sections were stained using routine H&E or Masson’s tri-chrome stain.

Masson’s Tri-Chrome

Tissue sections were analyzed with a light microscope and pictures taken using 20X, 40X, and 63X objective magnifications.

BAL ANALYSIS

BAL cells were spun onto slides and stained using HEMA 3 kit protocol.

RESULTS

40x Magnification

H&E

Masson’s Tri-Chrome

63x Magnification

H&E

Masson’s Tri-Chrome

FUTURE DIRECTIONS

- Infections caused epithelial necrosis and sloughing, and accumulation of mucopurulent discharge in the alveoli and bronchioles.
- Lesions were typical multifocal bronchiolitis with neutrophils, macrophages, and lymphocytes infiltration; capillary congestion, hemorrhage, extravasation of fluid and proteins, and formation of hyaline membranes (black arrows). Other changes were interalveolar septal thickening and alveoli collapse.
- Co-infecting RV1B with PR8 or PVM reduced disease severity as determined by lessened mortality and weight loss.
- Reduced inflammation in Balb/c mouse lungs co-infected with rhinovirus and influenza.

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