Stereological assessment of the intestinal morphology following Concanavalin A administration in mice

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Introduction
Concanavalin A (Con A) is a lectin derived from the jack bean plant (Canavalia ensiformis). In vitro Con A stimulates the release of a broad range of cytokines from lymphocytes and mononuclear cells and agglutinates red blood cells. In addition, Con A induces T cell activation. Intravenous administration of Con A induces a dose dependent acute liver injury in mice, which involves T cells and nonparenchymal liver cells, including Kupffer cells. Toll-like receptor-4 (TLR4) is a component of the innate immune system. It is a lipopolysaccharide (LPS) receptor. LPS is a major component of the outer membrane of the Gram-negative bacteria. The gut microbiota provides a substantial reservoir of LPS. Signalling via the LPS/TLR4 pathway induces expression of proinflammatory genes and production of cytokines. Previous studies have shown that Con A-induced injury is dependent on a functional TLR4 signalling pathway and that injury in response to Con A is ameliorated by depletion of Gram-negative bacteria in the gut (1). Furthermore, Con A induces increased gut permeability. We hypothesise that this increased gut permeability following Con A administration, leads to increased TLR4 signalling in the liver and this is essential for the development of liver injury in response to Con A. Therefore, we investigated the changes in the morphology of the intestinal lining following Con A administration in LPS-sensitive (C3H/HeN) and LPS-insensitive (C3H/HeJ, a mouse that is hyporesponsive to LPS) mice.

Materials and Methods
Con A (30 µg/g i.v.) was administered to C3H/HeN and C3H/HeJ mice. Intestinal tracts of were collected after euthanasia at 1, 2, 4 and 24-hour and from control mice (n=4/time-point). Histological sections from jejunum, ileum, caecum and colon-rectum were made according to Cavalieri method and vertical uniform random selections of stereological principles (2). Using these sections, the volumes (Fig. 1) and surface areas of muscularis, lamina propria, primary mucosa and villi of mucosa from each intestinal segments were estimated by applying stereological counting procedures.
Results and Discussion
The mean total volume of C3H/HeN jejunum smooth muscles, primary mucosa and villi showed an acute reduction 1 hour after Con A treatment. The volumes return towards normal at 4-hour however dropped 8 folds at 24-hour in comparison to C3H/HeJ. Surface area for jejunum remains consistent between these two groups. Ileum segments showed mean volume and mean surface area were two folds higher for C3H/HeJ. For colon analysis, mean volume showed similar result to ileum components however their surface areas were insignificantly different.

Conclusion
Stereological analysis demonstrated that the C3H/HeN volume of the jejunal primary mucosa and the villi were transiently reduced following Con A administration. Furthermore, we found that the C3H/HeJ ileum primary mucosa and villi surface area and volume were two folds higher than C3H/HeN. C3H/HeJ also showed higher colonic volume following Con A administration. These alterations to the structure of the gastrointestinal tract may lead to reduce barrier function in LPS-sensitive mice. It has been demonstrated that Con A-induced liver injury requires both a functional LPS/TLR4 signalling pathway and the presence of Gram-negative bacteria in the gut. Normal gut microbiota provide a substantial source of LPS. It has also been shown that Con A administration causes increased permeability in the gut. Our results suggest that Con A causes structural changes to the gut, in the jejunum, ileum and colon, leading to increased permeability. We hypothesise that these changes allow increased translocation of LPS from the gut, causing activation of LPS/TLR4 signalling and activation of this pathway is essential for the development of injury following Con A administration.

References