BSG 2016 - Abstract Submission

Inflammatory Bowel Disease

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ANTI-CD3 ANTIBODY INDUCES T-CELL MEDIATED APOPTOSIS AND SHEDDING OF MURINE SMALL INTESTINAL EPITHELIAL CELLS

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Introduction: Anti-CD3 antibody binds to the CD3/TCR complex on the wall of T lymphocytes and results in their activation. This leads to the release of various cytokines including tumour necrosis factor (TNF). TNF has previously been shown to bind to the TNFR1 receptor on the basolateral wall of small intestinal epithelial cells (IECs), triggering their apoptosis and shedding (Williams JM *et al*, DMM, 2013). We aimed to determine the time course and dose response of anti-CD3 antibody-induced apoptosis in murine small IECs and investigate whether this is regulated by the expression of members of the NFκB family of proteins.

Methods: Groups of 3 wild-type female C57BL/6J mice were injected intraperitoneally (i.p.) with 1mg/kg anti-CD3 antibody and were euthanased at different time-points from 1-6 hours. Groups of 3 wild-type female C57BL/6J were subsequently injected i.p. with 0.5-4 mg/kg anti-CD3 antibody and were killed after 1.5 hours. The responses of 6 $NF\kappa B1^{-/-}$, 6 $NF\kappa B2^{-/-}$ and 6 $c\text{-}Rel^{-/-}$ female mice were compared with 6 C57BL/6 mice 1.5 hours after i.p. administration of 2mg/kg anti-CD3 antibody. After euthanasia, the small intestine was dissected, fixed in formalin and paraffin embedded to produce histological slides. Immunohistochemistry was performed using a rabbit anti-mouse active caspase 3 primary antibody. Positively stained cells reflecting the percentage of shedding and apoptotic cells were scored on 20 villi per mouse on a cell positional basis. Data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA (with Tukey post-hoc test for multiple comparisons). $p<0.05$ was considered significant.

Results: 1mg/kg anti-CD3 antibody caused a significant increase in the percentage of IECs undergoing apoptosis and shedding from 1±0.1% in untreated mice to a peak of 2.3±0.1% at 1.5 hours then started to decline to reach 1.3±0.1% at 6 hours. The percentage of IECs undergoing apoptosis and shedding increased with increasing doses of anti-CD3 antibody with a maximum of 5.6±0.2% observed 1.5 hours after administration of 4mg/kg anti-CD3 antibody. Although $NF\kappa B1^{-/-}$ mice (5.1±0.3%) and $c\text{-}Rel^{-/-}$ mice (4.8±0.6%) showed more IEC apoptosis and shedding than wild-type (3.6±0.2%) these differences were not significant. However $NF\kappa B2^{-/-}$ mice were much more resistant (0.8±0.1%) ($p<0.001$). In all cases the effects of anti-CD3 antibody were most pronounced in the apical portion of small intestinal villi.

Conclusion: Systemic administration of anti-CD3 antibody induces apoptosis and shedding of murine IECs. The time course and cell positional distribution of apoptotic cells are very similar to those observed following administration of either lipopolysaccharide or TNF. The responses to anti-CD3 antibody are significantly affected by the expression of NFκB2.

Disclosure of Interest: None Declared