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Oral Presentations

O001 DISCOVERY OF A NOVEL TRANSCRIPTIONAL REGULATOR OF SUSTAINING TLR-MEDIATED INFLAMMATORY RESPONSES

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Introduction: Inflammatory genes induced by Toll-like receptor (TLR) activation show varying induction kinetics, with some genes strongly induced early after activation (like Nfkbiz), while others (like Il6) show delayed induction that is sustained for several hours [1]. This phenomenon is unexpected, considering the fact that many of the induced genes share common activating transcription factors such as NF-kB and AP-1 [2].

Methods: To investigate the underlying mechanisms, we carried out an RNAi screen using IL-6 as output, aiming to identify regulators involved in sustaining TLR-activated inflammatory gene induction.

Results: A novel regulator (Hit4) was identified and found to be specifically required to sustain the late inflammatory genes induced by TLR activation. RNAi knockdown of Hit4 in mouse bone-marrow derived macrophages led to decreased expression of the secondary response genes Il6, Edn1 and Lcn2, and the late primary response genes Il1a, Il1b, and Csf2, but did not affect induction of the primary response genes Nfkbiz, Egr1, and Egr2.

Most interestingly, Hit4 functioned specifically in sustaining rather than initiating the transcription, as Hit4 showed no effect on the early mRNA transcription before 2 h. We also find that Hit4 had no effect on interferon response genes, either induced by Lipid A (TLR4) or poly(I:C) (TLR3). In contrast, late inflammatory response genes induced by R848 (TLR7), Pam3CSK4 (TLR2), and Lipid A (TLR4) were all enhanced by Hit4. These data suggest that Hit4 is primarily involved in the regulation of MyD88- but not TRIF-mediated gene induction.

Further mechanistic investigation revealed that Hit4 functioned at the transcriptional level rather than post-transcriptional level, as Hit4 enhanced the level of nascent transcripts at later time points. This was further supported by the finding that this protein was required for recruitment of RNA pol II to the regulated gene loci at these times.

Moreover, Hit4 KO RAW264.7 macrophage cells failed to discriminate persistent and transient LPS stimulation. The KO cells failed to sustain the response by persistent LPS stimulation, which led to only low level of response similar to that in transient LPS stimulation. This suggests a role for Hit4 in discriminating brief spurious pulses of infectious signal from a more sustained real infection signal.

Conclusion: In summary, we have identified a novel transcriptional regulator functioning to sustain the transcription of late inflammatory genes. This protein could provide important new insight to the transcriptional mechanisms involved in sustaining the inflammatory response to persistent microbial signals.

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References

[1] M. Rabani, R. Raychowdhury, M. Jovanovic, M. Rooney, D.J. Stumpo, A. Pauli, N. Hacohen, A.F. Schier, P.J. Blackshear, N. Friedman, I. Amit, A. Regev, High-resolution sequencing and modeling identifies distinct dynamic RNA regulatory strategies, Cell 159(7) (2014) 1698–1710.
[2] O. Takeuchi, S. Akira, Pattern recognition receptors and inflammation, Cell 140(6) (2010) 805–820.

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O002 TRANSGENE EXPRESSION OF INTERLEUKIN-37 IN IL-10KO MICE INHIBITS COLON CARCINOGENSIS

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Introduction: Inflammatory bowel disease is associated with an increased risk of developing colon cancer. Interleukin (IL-) 37 is a fundamental inhibitor of innate immunity by reducing systemic and local inflammation. IL-37 exhibits intra- and extracellular functionality and we recently identified the IL-37 receptor composed of IL-18 receptor 1 and single Ig IL-1R-related molecule (SIGIRR). We showed that IL-37 reduces the LPS- or IL-1β-induced inflammatory response

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of immune cells and protects transgene mice against LPS-induced sepsis as well as in a model of acute colitis. IL-37 protein is expressed in healthy and diseased human bowel tissue and shows a similar expression pattern as IL-18. In the present study, we tested whether transgene IL-37 expression protects colon carcinogenesis secondary to chronic colitis in IL-10ko mice.

Methods: IL-37tg mice (C57Bl6) were crossbred with IL-10ko (C57Bl6) mice. Homozygous IL-10ko/IL-37tg mice were selected by genotyping. At 6 weeks of age mild colitis was induced by 2% dextran sulphate sodium (DSS) in drinking water for 5 days (IL-10ko n = 10, IL-10ko/IL-37tg n = 5). Subsequently, cyclooxygenase 2 inhibitor celecoxib (500 ìg/mouse) was applied by gastric gavage on day 7, 10 and 13 to trigger colon carcinogenesis as described (Glauben et al. Gut 2008). Mice were sacrified on day 171. Endpoints were clinical parameters as weight, rectal bleeding, stool consistency, colon length as well as cytokine response in LPS-stimulated whole blood and explanted colon cultures. Cytokine measurements were performed by Elisa (IL-6) and Bioplex assay (IL-1β, IL-10, IL-17, IFNγ, TNFα). Colon inflammation, number of adenoma/carcinoma and colitis-associated liver inflammation were analyzed by histology (HE staining).

Results: During the DSS-induction phase IL-10ko and IL-10ko/IL-37tg mice had a similar weight loss due to mild acute colitis. From day 115 there was a significantly improved weight gain in IL-10ko/IL-37tg mice. Colon length was similar in both groups. Whole blood assays showed similar basal cytokine levels. However, after LPS response, IL-10ko/IL-37tg compared to IL-10ko mice released less IL-6 ($p = 0.03$), IL-1β ($p = 0.03$), IFNγ ($p < 0.0001$), TNFα ($p < 0.003$), but more IL-10 ($p < 0.0001$) in supernatants of ex vivo stimulated whole blood. Ex vivo colon cultures showed a trend towards lower levels of pro-inflammatory cytokine production in IL-10ko/IL-37tg mice. Hemoglobin levels were higher in IL-10ko/IL-37tg mice ($p = 0.013$). All IL-10ko mice developed colon adenoma and carcinoma (5–10 adenoma/carcinoma per mouse). No adenoma or carcinoma were detected in the colon of IL-10ko/IL-37tg mice. Colitis-induced liver inflammation was markedly less in IL-10ko/IL-37tg mice. Detailed histological and immunohistochemistical analysis will be presented.

Conclusion: In conclusion, IL-37 transgene expression protects IL-10ko mice from colon carcinogenesis secondary to chronic colitis. Reduced pro-inflammatory immune responses and higher levels of anti-inflammatory IL-10 by IL-37 overexpression are central protective mechanisms. It remains unclear whether IL-37 has direct tumor suppressing properties.

Disclosure of Interest: None declared.

O003 EFFECTS OF MYCOBIOTA DYSBIOSIS DURING INTESTINAL INFLAMMATION

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Introduction: Although a complex community of fungi (so called mycobiota) resides in the gastrointestinal tract of various mammals, its contribution to the maintenance of intestinal homeostasis is poorly understood. This study examines how fungi can modulate intestinal inflammation.

Methods: We used a custom-developed database for the fungal "internal transcribed spacer" (ITS1) and a sequencing-based analysis to characterize the mycobiota of C57Bl6 mice along the digestive tract. The abundance of selected fungal species was validated by qPCR. Profiling of the bacterial microbiota was performed by 16S sequencing. The fungal and bacterial community were analyzed for composition, diversity (Shannon and Inverse Simpson indexes) using the phyloseq package in R. Beta diversity was calculated with the Bray–Curtis index using the vegan package. LDA Effect Size (LEfSe) was used to identify genera characterizing the different regions. The generalized linear models implemented in MaAsLin was used to find associations between the topology of the sample and microbiota composition. Network analysis was performed to detect co-occurrence and mutual exclusion at the genus level.

Colitis was induced by oral administration of DSS (in the drinking water for 7 days). The development of colitis was monitored by the weight loss and mortality rate. Immunophenotyping of lamina propria (LP) and mesenteric lymph node (mLN) and histology were performed at the end day (3 days after the withdrawal of DSS).

Results: We show that in contrast to bacterial community, where colonic and caecal bacteria microbiota clustered apart from other region, fungal communities clustered closely to one another. Interestingly, analysis at the genus levels revealed differences in the relative abundance among different regions, with the abundance of *Candida* spp. being higher in the murine colon. LefSE analysis of the murine esophagus and colon, showed that OTUs belonging to the *Candida* and *Saccharomyces* genus were significantly more abundant in the latter whereas *Nectariaceae* and *Sordariomycetes* were decreased. We therefore explored the effect of *Candida* spp. and *Saccharomycopsis fibuligera* on mucosal immunity and inflammation in the colon.

Our results show that, in the colon, administration of some fungal species, such as *Candida*, exacerbate the outcome of DSS colitis leading to a more pronounced weight loss and increased mortality rates. In contrast, other species, such as *S. fibuligera*, exert a protective effect.

Conclusion: Our results show that the composition of the intestinal mycobiota varies across the length of the intestine, with the *Candida* genus being more prevalent in the lower gastrointestinal tract. Our data suggest that specific fungi might be associated with site specific immune responses in the colon and might promote inflammatory conditions as a result of aberrant immunity to fungi.

Disclosure of Interest: None declared.

O004 MICROBIOME-DEPENDENT MODULATION OF MUCOSAL IMMUNITY AT THE OCULAR SURFACE

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Introduction: Mucosal sites that interface with the environment and provide barrier function include the intestine, nasopharynx, lung, female reproductive tract and the ocular surface. Disruption of immune homeostasis at the ocular surface is associated with discomfort, inflammation and potential loss of vision. Immune cells are present within the conjunctiva and can be affected by environmental factors, potentially including microorganisms. However, proof that a resident ocular microbiome exists and influences local immunity has been elusive. We used a mouse model of ocular surface disease to study whether commensal microbes are present in ocular mucosa and modulate immunity.