Research Proposal for CSC Scholarship Program

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Research Title: The role of gut microbiome derived Aryl hydrocarbon receptor (AhR) agonists in Inflammatory bowel disease (IBD).

Background and Aims

IBD, including ulcerative colitis (UC) and Crohn's disease (CD), is a chronic and relapsing inflammatory condition of the gastrointestinal tract [1]. IBD has become a global health burden affecting approximately 1 million Americans and 2.5 million Europeans [2]. The environment, diet, and lifestyle are widely recognized as potential triggers of IBD. Additionally, the etiology of IBD involves a complex interaction of genetic predisposition, environmental triggers, microbial factors, and immune responses. However, treatment of IBD can be complex and often unsuccessful due to an incomplete understanding of its pathogenesis and etiology. Therefore, elucidating the underlying molecular mechanisms of these risk factors of IBD to identify the targets and develop novel therapeutic strategies is necessary.

Despite the uncertainty regarding IBD's exact etiology, the gut microbiome has been implicated as a key factor [3]. For instance, faecal microbiota transplantation (FMT) exerts a beneficial effect on chronic intestinal inflammation by reshaping the gut microbiome of mouse models of IBD [4]. Our recent study found that faecal water from IBD patients induced higher DNA damage, and less protection against DNA damage in colonic epithelial cells [5]. A study on human gut microbiomes has highlighted the abnormal composition and functionality of gut microbiota in IBD, which have an impact on the immune system and host metabolism [6]. Moreover, the effect of gut microbiota suggested that interventions targeting the microbiome might be a potential path to treat IBD.

The gut microbiome can produce aryl hydrocarbon receptor (AhR) agonists. AhR is a ligand-activated transcription factor that was originally described as a molecular sensor for environmental toxins [7]. However, some studies found that AhR −/− mice are highly sensitive to DSS-induced colitis [8], while fecal samples from healthy subjects induce greater AhR activity than those from patients with IBD [9]. Moreover, accumulating evidence suggested that some AhR agonists such as tryptophan metabolites and butyrate produced by gut microbiota could reduce oxidative stress and control intestinal inflammation [10, 11]. Hence, AhR agonists derived from gut microbiota may have the potential as therapeutic targets for IBD.

In this study, we postulate that AhR agonists play a pivotal role during IBD treatment, and the gut microbiota is a key factor affecting IBD. This study will be conducted in IBD patients and healthy controls to determine the relationship between AhR agonists, IBD, and gut microbiota. Additionally, the *in vitro* fermentation experiment and of AhR

agonists with IBD patients' fecal sample will be used to screen out the optimal AhR agonist and verify if changes in the gut microbiome affect the development of IBD.

Novelty

In this study, human biological samples and cell models are used to investigate the role of AhR agonists in the development of IBD. In addition, we will analyze the gut microbiota to examine the relationship between the AhR agonists that are formed by the gut microbiome and how these affect subsequent chronic inflammation and DNA damage. The study may shed light on how dietary and gut microbiota interact to modulate the etiology of IBD through modulating the formation of AhR agonists. Based on the result of this study, natural or synthetic AhR agonists may become part of IBD treatment regimens.

Approach

The role of AhR agonists in IBD and gut microbiota will be studied at the department of Pharmacology & Toxicology at Maastricht University, in collaboration with the department of gastroenterology of the Maastricht Medical Centre.

Methodology

The experiments performed in this project will have 2 experimental arms:1. *in vitro* fermentation of AhR agonist and IBD patients faecal and *in vitro* cell model that mimics intestinal inflammation to screen out the optimal AhR agonist (point 1-3), and 2. Gut microbiota analysis of samples from IBD patients to evaluate the efficacy of AhR agonist (point 4-6)

1. Fresh faecal samples obtained from IBD patient will be mixed with Modified peptone-yeast extract glucose (MPYG) medium in an anaerobic station to prepare a faecal suspension. AhR agonists will be co-cultured with faecal suspension in anaerobic environment for 48hrs. Then the fermentation broth will be collected for 16S rRNA sequencing to compare the structural changes of gut microbiota.

2. Human intestinal epithelial cell line HT29 cells will be co-cultured with the supernatant of fermentation broth obtained from point 4. After 24 hrs of co-culture, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) will be added to evaluate the cell viability. Trans epithelial electrical resistance (TEER) will be used to test barrier integrity of intestinal epithelial cell.

3. Add TNF-α to HT29 cells cultural medium to mimic intestinal inflammation and co-culture with supernatant of fermentation broth. After 6 hrs of co-culture, supernatant of cell culture will be collected to detect the level of IL-22 with the ELISA kit.

4. Biological samples of IBD patients and healthy controls will be acquired from the biobank of the departments of gastroenterology and surgery of the Maastricht Medical Centre. We will analyze 10 samples in 5 groups (CD, CD with addition of AhR agonist,

UC, UC with addition of AhR agonist, healthy controls, healthy controls with addition of AhR agonist).

5. Histopathology will be performed to evaluate the therapeutic effect of AhR agonists on IBD patients. AhR and its downstream target, CYP1A1 expression level will be accessed in colonic samples obtained from IBD patients, IBD patients with AhR agonist, and healthy controls. These data will be linked to dietary and clinical data that are available from these patients, including gender, type of IBD and stage of disease.

6. The composition of gut bacterial communities of IBD patients, IBD patients with AhR agonist, and healthy controls will be determined by 16S ribosomal RNA (16S rRNA) sequencing. The 16S rRNA sequencing data will be processed with closed reference Operational Taxonomic Units (OTU) picking. After generating the OTU tables, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) will be used to predict microbial community metagenomes. Spearman's correlation analysis will be performed on the genus or species associated with clinical indices to find out the key probiotic that might be increased by AhR agonist treatment.

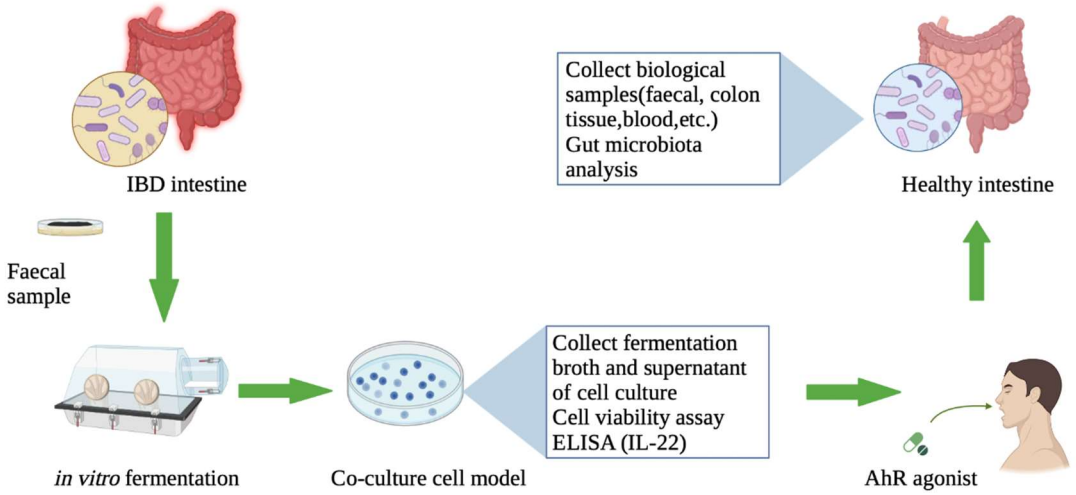

Figure 1. Graphical Abstract of this project
Biological samples obtained from IBD patients, IBD patients treated with AhR agonists, and healthy controls will be detected to assess the effectiveness of treatment. *In vitro* fermentation experiment and inflammation intestine cell model will further identify the the role of AhR agonist in IBD and gut microbiota.

Project Milestones

Time / Tasks	2023.9-2024.8	2024.9-2025.8	2025.9-2026.8	2026.9-2027.6
Samplecollection &Experimentspreparation				
Literaturereview				
Conducting experiments				
Data analysis				
Writing papers				
Submitting papers				
Writing Thesis				

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