Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 2471; doi:10.3390/ijms19092471

Review

Advances in *in-vivo* Imaging of Inflammatory Bowel Disease mediators

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Abstract:

Inflammatory Bowel Disease (IBD) is characterized by chronic remitting and relapsing inflammation of the lower gastrointestinal tract. The etiology underlying IBD remains unknown but is thought to involve a hypersensitive immune response to environmental antigen, including the microbiota. Diagnosis and monitoring of disease is heavily reliant on endoscopy, which is invasive and does not provide information regarding specific mediators. This review describes recent developments in imaging of IBD with a focus on PET and SPECT imaging of inflammatory mediators, and how this may be applied to the microbiota.

Keywords: Inflammatory Bowel Disease (IBD), colitis, PET, SPECT, microbiota, cytokine, chemokine, inflammation

1. Introduction

Inflammatory Bowel Disease (IBD) is an inflammatory disorder of the gastrointestinal (GI) tract which has a substantial impact on quality of life. The causes of inflammation in IBD remain unknown but are thought to involve a hypersensitive immune response to the intestinal microbiota. IBD is commonly associated with dysbiosis, but it remains unknown whether dysbiosis is a cause or consequence of inflammation. Diagnosis and monitoring of IBD is heavily reliant on endoscopy,

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which is an invasive technique that requires bowel preparation and typically also anesthesia. Although generally well tolerated, endoscopy impacts substantially on quality of life of patients and does not provide any direct information regarding the role that specific mediators contribute toward inflammation. Therefore, new technologies are required that are sensitive enough to grade disease severity, can be optimized for detection of specific mediators and are non- or only minimally invasive. Recent developments in PET imaging, and particularly the use of antibody conjugates, have demonstrated success in cancers, but are only recently being adapted to studies of IBD. This review summarizes the evolution of these technologies in IBD studies, how they have been used to detect specific mediators of inflammation, and their potential to image microbiota.

2. Inflammatory Bowel Disease

IBD is a collection of debilitating idiopathic diseases characterized by chronic inflammation of the lower gastrointestinal tract that have a remitting and relapsing disease course. The global incidence of IBD is estimated to be 0.3% and is widely regarded as increasing [1]. The cause(s) of IBD remain unknown, however are thought to involve aberrant immune responses to environmental stimuli in people with a complex genetic predisposition. The relatively recent increase in the incidence of IBD is perplexing, but potentially highlights the importance of environmental factors in its etiology. The two major subtypes of IBD are Ulcerative Colitis (UC) and Crohn's Disease (CD) which can typically be distinguished by pathological and histological differences. Inflammation in UC is typically restricted to the mucosal layer of the colon and progresses in a contiguous manner. CD differs in that it is characterized by transmural skip lesions that can occur anywhere in the GI tract but are typically located in the ileum with and without involvement of the colon. The initiating factors and

52 inflammatory aspects of UC and CD are likely to differ given the distinctions between their pathology
53 but are only poorly understood. Much more is known about how inflammation is perpetuated in
54 IBD, and this has led to the relatively recent development of biologic drugs targeting specific immune
55 mechanisms. However, treatment gaps remain in IBD due to intolerance, incomplete efficacy and
56 side effects of current therapies, and the disease course in both UC and CD is characterized by
57 variable periods of remission and relapse. Prognostic indicators of relapse in IBD are poor, requiring
58 patients to undergo constant surveillance. Severe persistent or relapsing symptoms may lead to
59 surgery [2] which can be curative for UC (withstanding complications), but is not curative for CD.
60 This high rate of surgery highlights the ongoing need for a deeper understanding of the mechanisms
61 involved in initiating and perpetuating inflammation in IBD.
62
63 **2.1 Imaging IBD: Current approaches**
64 The treatment of IBD depends on clinical severity which is currently highly dependent on endoscopy,
65 where a thin flexible tube with a camera on the end is moved through the lumen. Endoscopy directly
66 images the gastrointestinal mucosa for assessment of disease stage and monitoring and, when forceps
67 are attached, allows for the collection of biopsy material for analysis of pathology and mechanistic
68 studies. Endoscopy can differentiate between UC and CD in the majority of cases [3]. However,
69 endoscopic approaches have limitations as the quality of results is operator dependent and may not
70 be sensitive enough for assessment of clinical severity. Furthermore, endoscopic imaging is restricted
71 to the superficial mucosal layers of the intestine and therefore provides no information regarding
72 inflammatory damage to the deeper layers of the intestinal wall, the degree of muscle thickness or
73 the diameter of the lumen. Finally, a major limitation of endoscopy is the difficulty in reaching the

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74 small intestine due to the distance needed to be covered and the complexities of intestinal anatomy
75 with its multiple loops and folds.
76
77 A number of novel imaging technologies are emerging that complement or may even replace
78 endoscopy as the gold standard for clinical diagnosis, as recently reviewed [4]. Chromoendography
79 involves the application of dyes onto the mucosa, improving endoscopic characterization of lesions
80 and neoplasia and potentially correlating with histological damage, although the latter remains
81 controversial [5]. Confocal laser endomicroscopy and endocytoscopy combine high resolution
82 microscopic imaging with endoscopy and again are primarily used to detect colonic dysplasia and
83 neoplasia [6]. Wireless capsule technologies may circumvent the limitations that endoscopy has in
84 accessing difficult to reach areas of the small intestine. While these capsules provide high resolution
85 imaging, they have similar limitations to standard endoscopy regarding assessment of inflammatory
86 damage to the deeper layers of the colon wall, but may also require surgery for removal when
87 stricturing occurs [7]. Less invasive imaging technologies include barium X-rays, Magnetic
88 Resonance Imaging (MRI), Computed Tomography (CT), positron emission tomography (PET) and
89 single photon emission computed tomography (SPECT). Barium enemas provide excellent
90 visualization of the bowel and can reveal thickening of the bowel wall but is not recommended in
91 patients with severe inflammation due to the risk of complications such as toxic megacolon. MRI, CT,
92 PET and SPECT are minimally invasive relative to endoscopy and offer the additional benefits of
93 visualizing planes through the colon wall and extra-intestinal manifestations. It is important to note
94 that while these technological advances are welcome for IBD management, none of them currently
95 target specific mediators. This highlights the need for the development of new imaging technologies

that are highly sensitive, quantitative and able to provide longitudinal data in real time but are also only minimally invasive. Recent advances in PET and SPECT imaging have the potential to cover all these issues.

3. Imaging inflammatory mechanisms in IBD: which targets to choose?

3.1 Immune targets

Inflammation is central to IBD however much remains to be understood regarding the causes of inflammation in human IBD. Animal models of colitis have significantly improved our understanding of how inflammation develops and perpetuates, highlighting roles for both the innate and the adaptive arms of the immune response. It is currently thought that inflammation in IBD is driven by a loss of immune tolerance to autologous proteins in the colon wall and foreign antigen in the lumen, including the microbiota. This initiates a cascade of cytokines and chemokines which cause an influx of immune cells. Immune cells are attracted to sites of inflammation by chemokines and also by upregulated expression of 'gut-homing' integrins on the cell surface, including $\alpha_4\beta_7$ [8]. Subsequent inflammatory lesions and damage to the epithelial wall and deeper layers of intestinal tissue result in production of damage related mediators during active disease, which progresses toward mediators related to tissue-remodeling and wound healing as disease progresses toward remission, but also toward fibrosis after repeated or extended periods of inflammation. The immunological basis for the differences in pathophysiology of UC and CD are not clear, however it is suggested that CD is driven by a T_H1 / T_H17 type immune response while is thought to be mediated by a T_H2 type immune response (↑ IL-5, IL-13) that is atypical as IL-4 is not involved.

Recent advances in two-photon laser scanning microscopy have enabled *in-vivo* imaging of deep visceral tissues and immune cells in particular. This technique was elegantly used to identify goblet cell associated passage (GAPs) as the major mechanism for delivering luminal antigen to dendritic cells in the small intestine in health, but only appear in the colon after microbe sensing pathways were disrupted [9, 10]. These findings potentially highlight a role for GAPs in IBD, however they are yet to be investigated in the IBD setting. We have recently applied two-photon laser scanning microscopy to investigate immune cell migration in the tri-nitro benzene sulphonic acid (TNBS) model of colitis, demonstrating *in-vivo* that not only are immune cells in very close apposition to nerves within the colon wall, but also that myeloid cell infiltration is increased in TNBS colitis [11]. This technique can be easily combined with reporter mice and promises to increase the understanding of the mechanisms involved in immune cell migration from blood vessels into the lower GI tract in IBD.

3.2 Microbiota

Recent improvements in high throughput 'omics' technology have also revealed the involvement of the microbiome in IBD. The general consensus indicates that active IBD is characterized by an overall loss of diversity coupled with a dysbiotic phenotype primarily depicted by elevated *Proteobacteria* and decreased *Fermicutes*, although inconsistencies between studies remain [12-14]. Functionally, the major classes of bacteria affected relate to the production of short-chain fatty acids (e.g. *Faecalibacerium prausnitzii*), bacteria with mucolytic activity (e.g. *Ruminococcous torques*) and sulfate reducing species including *Desulfovibrio* [15-17]. While this dysbiotic composition may contribute to disease progression through dysregulated epithelial barrier function and aberrant immune signaling,

it is currently contentious whether dysbiosis itself causes IBD or is simply an effect of the disease course [18].

Uncovering the compositional shifts in the microbiome has undoubtedly increased our understanding of its role in disease initiation, progression and relapse. However, techniques such as 16S pyrosequencing are inherently limited by the variations that exist in aspects of their methodology including inconsistent or incomplete reference libraries, the unknown impact of batched analyses, non-standardized statistical analyses, and an inability to adequately appreciate the heterogeneity in the microbiome [19]. This is further confounded by the fact that the annotation is based on putative association of the 16S rRNA gene with a taxon defined as an operational taxonomic unit (OTU) [20, 21]. Although whole genome shotgun sequencing has enhanced our ability define species taxa with more accuracy, it remains an expensive alternative to 16S and requires significantly greater data analysis [19]. As such, conclusions regarding the functional implications of compositional shifts remain difficult to draw, relying on metagenomics inference using phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt). Non-the-less changes in microbiota composition offer the intriguing potential to image microbiota as markers of disease severity in IBD.

4. PET and SPECT imaging of the gastrointestinal tract in IBD

PET studies have traditionally relied on the use of ^{18}-fludeoxyglucose (^{18}F-FDG), a radiolabeled glucose analogue that detects tissue glucose metabolism. This can be useful for detecting sites of inflammation but does not offer any information regarding the involvement of specific mediators. Radiolabeled leukocytes have been the gold standard for nuclear imaging of GI inflammation for

decades due to accessibility from both supply and economical ends. SPECT imaging of ^{99Tm} -leukocytes was shown nearly two decades ago to accurately assess the extent and severity of people with severe UC, correlating with endoscopic and histological findings [22]. However, limitations with radiolabeled leukocytes exist, including a relatively extensive protocol required for working up the cells, risks of cross-contamination and, importantly, the non-specific nature of the tracer. These limitations have spurred interest in developing new technologies for imaging, with antibodies in the prime place. Multiple clinical trials are currently utilizing radioisotope labelled antibodies for immunoPET in cancer to tailor personalized therapies based on specifically targeted diagnostic tests [23, 24]. However, imaging of IBD is presently limited to pre-clinical studies.

4.1. Imaging colitis in animal models

Monoclonal antibodies have exquisite selectivity for their targets which has been exploited clinically for decades. Nuclear imaging of colitis has so far relied upon two models, the TNBS model and the dextran sulfate sodium (DSS) model. TNBS is combined with ethanol to break the mucosal barrier and administered by enema, and inflammation is therefore restricted to the colon. TNBS is a hapten; it binds to autologous proteins and luminal microbiota turning them immunogenic. The colitis that develops is transmural, involves an IL-12 driven T_H1/T_H17 type response and is therefore considered to model CD. DSS differs as it is directly toxic to epithelial cells and, when administered by the oral route in drinking water, results in a colitis that takes longer to develop than TNBS. DSS colitis is considered to model UC as the colitis results from direct damage to the epithelial layer and is not as deep as that which occurs with the TNBS model. While animal models are useful for emphasizing the importance of specific mediators and mechanisms, it is important to note that findings from

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184 animal models can be difficult to translate to human IBD. This is common with most chronic diseases
185 that have a remitting and relapsing course due to the involvement of multiple and overlapping
186 pathways that are inter-twined with disease stage as inflammation progresses and regresses,
187 highlighting the requirement for extensive validation in human subjects.
188
189 4.1.1. PET and SPECT imaging of **immune mediators** in colitis
190 Cell-secreted immune mediators are generally classified as chemokines or cytokines, with
191 chemokines mediating cell attraction and cytokines mediating immune responses. Table 1 provides
192 an overview of PET/SPECT studies of immune mediators in human IBD and animal models of colitis.
193 The first study investigating whether radiolabeled antibodies are efficacious for imaging colitis was
194 disappointing as ^{111}In labelled -IgG uptake was substantially lower than ^{111}In labelled -leukocytes, the
195 gold standard at the time, and also the newly developed ^{111}In labelled -liposomes in TNBS colitic
196 rabbits [25]. While imaging of liposomes and leukocytes enabled grading of inflammation, the
197 contrast provided from IgG labelling was not sufficient for this to occur. This result was not
198 surprising given the large abundance of IgG in circulating blood and its relative non-selectivity for
199 colitis. Interleukin (IL) -8, more recently renamed CXCL8, is a chemokine produced by innate
200 immune cells but also cells in other tissues including epithelial cells and endothelial cells. IL-8 is
201 primarily involved in attracting neutrophils to inflammatory sites, but also attracts other
202 granulocytes including mast cells. Colonic inflammation in TNBS treated rabbits was detected more
203 readily with ^{99m}Tc labelled -IL-8 than ^{99m}Tc labelled -granulocytes [26]. Furthermore, the severity of
204 inflammation was also able to be graded with ^{99m}Tc-IL-8, but not with ^{99m}Tc-granulocytes. More
205 recently, IL-8 was again labelled with ^{99m}Tc for SPECT studies in a large clinical study of people with

206 IBD and was found to have higher sensitivity for detecting inflammation than endoscopy, although
207 specificity was lower than endoscopy [27]. TNF-α is a cytokine that is secreted by a range of immune
208 cells from both the innate and adaptive arms of the immune response. TNF-α is produced in large
209 amounts in many autoimmune diseases, including IBD. Neutralizing TNF-α with antibodies, for
210 example Infliximab, has proven success in treating IBD [28, 29]. In pre-clinical studies, ^{99m}Tc labelled
211 -Infliximab was able to discriminate between moderate and severe colonic inflammation in TNBS
212 treated rats [30]. However, enthusiasm for Infliximab as a tracer in preclinical studies was hampered
213 by high background uptake in healthy control rats. This study is of importance as it not only revealed
214 TNF-α mediated inflammation, but it also demonstrated target specificity of a clinically useful drug
215 for IBD. This is also particularly significant given the increased development of biologics, particularly
216 antibody-based therapies, but also the relatively recent expansion of personalized medicine and
217 theragnostic approaches toward treating disease.
218
219 4.1.2. Imaging **immune cells** in colitis
220 The DSS colitis model is the most commonly used model to image immune cells in colitis. Table 1
221 provides an overview of PET/SPECT studies of immune cells in human IBD and animal models of
222 colitis. The integrins α_4 and β_7 form a heterodimer ($\alpha_4\beta_7$) which specifically directs the migration, or
223 homing, of immune cells to the gastrointestinal tract via interactions with its ligand the addressin
224 MAdDCAM-1 [8]. Targeting this pathway has proved successful in IBD with the recent development
225 of Vedolizumab, a monoclonal antibody that blocks $\alpha_4\beta_7$ [31]. Uptake of ^{64}Cu labelled -β_7 antibodies
226 was increased in DSS colitis relative to healthy controls and to ^{64}Cu labelled -IgG isotype negative
227 control [32]. However, high levels of background were also observed in non-target organs including

the small intestine and stomach, prompting the development of antibody fragments. Uptake of ^{64}Cu labelled -β_7 antibody fragments was superior to both whole ^{64}Cu labelled -β_7 antibodies, but also to ^{64}Cu labelled -$\alpha_4\beta_7$, suggesting that antibody fragments may provide enhanced signaling of inflammation in IBD than whole antibodies [33]. The concept of increased sensitivity with small antibodies has recently been validated in cancer biology and in inflammation. ^{18}F labelled antibody fragments directed against the antigen presenting complex MHCII were much more sensitive than standard ^{18}F-FDG in detecting xenoplanted tumors, even detecting tumors when growth was neither palpable or visible [34]. Furthermore, ^{18}F- labelled antibody fragments directed against CD11b detected complete Freund's adjuvant (CFA) induced inflammation in the mouse paw much earlier than standard ^{18}F-FDG [34]. Most recently, ^{89}Zr labelled antibody fragments against the T$_{HELPER}$ marker CD4 were observed to have higher uptake in the colon of DSS mice compared to health [35]. However, uptake was also increased in the spleen and lymph nodes, highlighting the intrinsic difficulties in imaging immune cells during inflammation as they generally migrate to the site of inflammation from distant lymph nodes.

4.2 Imaging the **microbiota** in colitis

As our understanding of how microbiota and host interact grows, it is necessary to move beyond current genomics technology and compositional descriptions. This remains a daunting task when imaging specific microbiota in the gut as it is limited by the diversity of materials that need to be maintained, which is of particular importance when considering the sensitivity of the mucous layer to standard fixation methods and the heterogeneity within gut microenvironments. As such, it is critical that a robust protocol including mucous-preserving sample preparation, image segmentation

and quantitative analysis tools be developed. A framework satisfying these criteria was recently developed by Earle et. al. [36], where high-resolution quantification of spatial organization of the gut microbiome revealed that changes in the proximity of microbes to the epithelium are sufficient to induce increased expression of key inflammatory markers despite negligible shifts in microbiota composition. Their protocol included integrating formalin-free methacarn preservation with fluorescent in-situ hybridization and, most importantly, high-end software analysis called BacSpace. BacSpase enables 1) stitching of overlapping images into one continuous image, 2) landmark definition, 3) differentiation of background autofluorescence (e.g. diet-derived plant material, shed epithelial cells, blood and 4) measurement of cell-cell and cell-landmark distance distributions based on a well-defined local coordinate system, revolutionizing the analysis and interpretation of microbiome related data. Most recently, chemical imaging techniques exploiting a blinking Surface Enhanced Raman Spectroscopy (SERS) have been used to visualize bacteria in-vitro with exquisite resolution, and the ability to differentiate between the chemical signatures of different bacteria [37].

Several minimally invasive techniques have also been applied to investigate the activity of bacteria *in-vivo* including MRI, CT, fluorescence / bioluminescence imaging and PET, however the majority of these only provide indirect information about bacterial activity by inference from immune function [38, 39]. Recent advances exploiting the bacterial uptake of carbohydrates have proven successful for the selective *in-vivo* imaging of bacteria independently of host factors and secondary pathologies [39, 40]. Furthermore, specific bacteria have also been labelled with iron oxide nanoparticles for MRI imaging to track bacteria longitudinally during infection [38]. These developments highlight the potential to image specific bacteria during inflammation but are yet to be applied to IBD.

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5. Limitations of radiolabeled antibodies

Radiolabeling antibodies or immune mediators offers the real potential for *in vivo* diagnostic imaging however several issues remain that currently limit enthusiasm for widespread use. The primary limitation is economical-based, as specialized equipment is required for the manufacture and detection of radiolabels. These costs are high, however may be mitigated by improved clinical outcomes including early detection and potential the potential development of personalized medicines to improve clinical responses and reduce side effects. Limitations related to biology also exist, particularly with relation to the relatively long half-life of antibodies and the time they require to move through the circulatory system to the site of inflammation. This relates to the increased risk of cancer due to radiation exposure, particularly as IBD patients already have a higher risk of colon cancer. However, PET is considered safe and the effective dose of whole body ^{18}F-FDG was approximately 7 mSv, much less that the 16 mSv used for a routine abdominal-pelvic CT with contrast [41, 42]. Efforts are underway to reduce circulation time by using pre-targeted methods, as explained in more detail below. The relatively large structure of antibodies may lead to difficulties in penetration of the site of inflammation, although this may be mitigated by altering the structure of the antibody, also explained in more detail below. A limitation also relates to the single mode of PET detection; only a single mediator can be imaged. This is problematic in terms of detailed studies of immune cell subsets as they are typically identified by flow cytometry techniques requiring multiple targets. Furthermore, intracellular cytoplasmic cytokines and nuclear transcription factors are increasingly being used to characterize immune cell subsets, however this typically requires cell permeabilization which is not possible *in-vivo*.

294

295 **6. Future directions for imaging IBD**

296 Remarkable progress has been achieved in recent years toward the understanding of IBD and its
297 underpinning disease mechanisms. This has resulted in a wide array of specific biologics for
298 treatment, but they are yet to be applied to diagnosis or surveillance. The complex immunological
299 mechanisms of IBD indicate that there are multiple markers that could potentially be used for
300 diagnostic imaging on the one hand, and drug bio-availability at the inflammatory site on the other.
301 Imaging specific sites of inflammation should be achievable relatively easily, however determining
302 markers that differentiate between IBD and other gastrointestinal disease, such as cancer, is more
303 problematic and requires extensive pre-clinical validation.

304

305 Intact antibodies are increasingly used as therapeutics, however their utility as imaging agents is
306 reduced by their extended half-life as the duration of time required to reach a sufficient signal to
307 noise ratio is long. Furthermore, extended half-lives may also lead to other unwanted biological
308 activity and side effects, which may alter the biological function being imaged. Recent developments
309 in antibody engineering include a shift from whole IgG antibodies to fragments which enhances their
310 physical properties, leading to enhanced efficacy as extensively reviewed in [43]. Enzymatic cleavage
311 or restructuring of the antibody attenuates the problems associated with half-life by decreasing
312 clearance times. Furthermore, the decreased amount of time required to reach a suitable signal also
313 allows for the use of short-lived isotopes decreasing radiation exposure.

314 Pre-targeting strategies have also emerged as a means of improving efficacy whilst reducing
315 radiation dose. Cook et al (2017) describe a novel approach for pre-targeted PET based on a

316 biorthogonal inverse electron demand Diels-Alder reaction between a ^{64}Cu-labelled radioligand and
317 a site specific immunoconjugate. This occurs in four steps: injection of the modified antibody,
318 accumulation of the antibody at its target site and clearance from the blood, 24 hours later the
319 injection of the radioligand and the *in vivo* click ligation between the immunoconjugate and
320 radioligand and subsequent excretion of excess radioligand. Target tumor tissue was able to be
321 delineated four hours post injection of the radioligand, with image contrast improving with time with
322 an ideal sound to noise ratio at 24 hours. This pre-targeting approach and similar approaches may
323 expedite clinical translation of future immunoconjugates for imaging.
324
325 A tantalizing approach is the potential to image different bacterial species, however is currently again
326 limited by the lack of knowledge of functional roles for specific microbiota in IBD on the one hand,
327 and the limited ability of PET to simultaneously label multiple targets. Furthermore, imaging of
328 microbiota in general currently requires models where the bacteria can be isolated, labelled and re-
329 administered. Future developments including the ability to use multiple labels, but also advances in
330 the identification of bacterial species of interest and anaerobic culture of microbiota would be a
331 welcome addition for preclinical models of IBD in the first instance before clinical studies are
332 warranted. Furthermore, the potential to exploit factors that are selectively produced by bacteria and
333 differentiate between active and quiescent states provides an intriguing insight. Bacterial toxins may
334 be the ideal sensor, as they are typically only secreted during disease, readily identifiable by
335 antibodies and relatively easily attached to various detection technologies. However, biosensing
336 technology remains limited in its ability to provide insight into the spatial organization of the

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337 microbiome, and as such the ideal microbial analysis tool remains elusive and current investigations
338 must combine genomic, visual and activity-based measures.
339
340 **7. Summary:**
341 Imaging has a critical role in the diagnosis and management of IBD patients. Endoscopic studies are
342 currently the gold standard. However, the clear need for new technologies that are less invasive than
343 endoscopy. Animal studies indicate that PET and SPECT imaging of immune cells and / or mediators
344 may be useful for the management and diagnosis of IBD. However, translation of these technologies
345 into human studies is currently lacking, despite progress in this area in other diseases including
346 cancer. Numerous immune cells and mediators are potential targets for PET / SPECT imaging of
347 active IBD, however options for imaging of microbiota are limited by the lack of understanding of
348 the role specific microbiota contribute toward IBD.
349
350

Author Contributions: ND, HRW and PAH conceptualized, wrote and edited the review.

Funding: This research was funded by a University of Australian Postgraduate Award and a William T Southcott Nuclear Medicine Scholarship to ND, a NHMRC C.J. Wright Biomedical Fellowship to HRW and a NHMRC R.D. Wright Biomedical Research Fellowship to PAH.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

^{18}F	^{18}Fluorine
^{89}Zr	^{89}Zirconium
^{99m}Tc	^{99m}Technetium
^{111}In	^{111}Indium
CD	Crohn's Disease
CD4	Cluster of differentiation 4
CFA	Complete Freund's Adjuvant
CT	Computed Tomography
CXCL8	C-X-C motif chemokine ligand 8; interleukin 8
DSS	Dextran sodium sulfate
GAP	Goblet cell associated passage
GI	Gastrointestinal
GIT	Gastrointestinal tract
Fab	Fragment antigen-binding
HMPAO	Hexamethylpropyleneamine oxime
HYNIC	Hydrazinonicotinic acid
IBD	Inflammatory bowel disease
IgG	Immunoglobulin G
IL	Interleukin
MAdCAM-1	Mucosal vascular addressin cell adhesion molecule 1
MHCII	Major histocompatibility complex II
MRI	Magnetic resonance imaging
mSv	Miliseiverts
OTU	Operational taxonomic unit
PET	Positron emission tomography
rRNA	Ribosomal ribonucleic acid
SERS	Surface enhanced Raman spectroscopy
SPECT	Single photon emission computed tomography
TNBS	Tri-nitro benzene sulfonic acid
TNFα	Tumor necrosis factor alpha
VHH	Variable domain of heavy chain antibodies
WBC	White blood cell

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Target	Tracers	Species	Model	Outcome	Reference
Leukocytes	^{99m}Tc-HMPAO-leukocytes	Humans	UC	+	[22]
CXCL8	^{99m}Tc-CXCL8	Humans	CD and UC	+	[27]
β_7	^{64}Cu-FIB504.64-Fab	Mice	DSS	+	[32]
$\alpha_4\beta_7$ β_7 β_7	^{64}Cu-DATK32 ^{64}Cu-FIB504.64-Fab ^{64}Cu-FIB504.64-F(ab')$_2$	Mice	DSS	+ + ++	[33]
CD4	^{89}Zr-GK1.5 cys-diabody	Mice	DSS	++	[35]
TNFα	^{99m}Tc-Infliximab	Rats	TNBS	+	[30]
IgG Leukocytes Liposomes	^{111}In-IgG ^{111}In-WBC ^{111}In-liposomes	Rabbits	TNBS	- ++ +	[25]
IL-8 Granulocytes	^{99m}Tc-HYNIC-IL-8 ^{99m}Tc-HMPAO-Granulocytes	Rabbits	TNBS	++ +	[26]

454 **Table 1**: **Summary of PET / SPECT imaging of human IBD and animal models of colitis.** - Not
455 suitable for colitis imaging, + satisfactory image quality, ++ excellent image quality.