The Molecular Biology of Inflammatory Bowel Diseases

Anthony P. Corfield*1, Heather M. Wallace† and Chris S.J. Probert‡

*Mucin Research Group, School of Clinical Sciences, Bristol Royal Infirmary, Level 7, Marlborough Street, Bristol BS2 8HW, U.K., †University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, U.K., and ‡Clinical Gastroenterology Research, School of Clinical Sciences, Bristol Royal Infirmary, Level 7, Marlborough Street, Bristol BS2 8HW, U.K.

Abstract

IBDs (inflammatory bowel diseases) are a group of diseases affecting the gastrointestinal tract. The diseases are multifactorial and cover genetic aspects: susceptibility genes, innate and adaptive responses to inflammation, and structure and efficacy of the mucosal protective barrier. Animal models of IBD have been developed to gain further knowledge of the disease mechanisms. These topics form an overlapping background to enable an improved understanding of the molecular features of these diseases. A series of articles is presented based on the topics covered at the Biochemical Society Focused Meeting The Molecular Biology of Inflammatory Bowel Diseases.

The IBDs (inflammatory bowel diseases) are mainly represented by UC (ulcerative colitis) and Crohn’s disease, but also have peripheral interactions with other gastrointestinal disorders. IBD remains a major gastrointestinal healthcare issue. Improved understanding of disease mechanisms has always been a central aim of IBD studies, but has remained difficult due to the different forms of disease and the multifactorial background of disease patterns. The majority of data available has been focused in the clinical areas with related pathological, endoscopic, microbiological and radiological assessments. The last few years have seen significant developments in molecular approaches to mucosal disease impinging on disease mechanisms in IBD [1]. Progress has been reported in three linked areas of study. The genetics of IBD has revealed a number of susceptibility genes which are linked directly with the integrity and efficiency of the mucosal barrier and interaction through both the innate and adaptive immune systems. Identification of the commensal microflora as a player in the gastrointestinal environment leading to abnormal immune response in the gut during IBD has opened a second area of work. Both of these aspects have been backed by mouse models of IBD which have made major contributions to disease mechanisms in IBD.

The Biochemical Society’s Focused Meeting in March 2011 assembled a group of speakers covering pathogenesis, immunology, microflora, nutrition, genetics, biomarker screening and animal models associated with IBD.

IBD pathogenesis was reviewed by Derek Jewell (Oxford). It has evolved through many stages since the initial identification of UC by Hurst in 1921 [2] and Crohn’s disease by Crohn, Ginsberg and Oppenheimer in 1932 [3]. Links with microflora have been a feature of study for many years, but this remains a controversial issue. Studies with Mycoplasma paratuberculosis by Thayer et al. [4] and Hermon-Taylor and co-workers [5] preceded metabolomic studies of urine samples, allowing the identification of different associated microbiota when UC was compared with Crohn’s disease [6].

Study of aetiopathogenesis, considering the interaction of genetic traits with the environment through meta-analysis of genome-wide association with large numbers of IBD patients (N>6000) compared with controls (N>15,000), generated more than 100 relevant genes for Crohn’s disease and >50 for...
UC, with ~50 shared between them (reviewed by Jack Satsangi, Edinburgh). Focus on genes from several areas accumulated. Under the adaptive immune response, HLA class 2, IL10 (IL is interleukin), IL12, IL17, IL23 and IL23R (IL-23 receptor) related to T-cell function; the innate immune response linked NOD2 (nucleotide-binding oligomerization domain 2), TLR4 (Toll-like receptor 4) and NALP3 (NLR (NOD-like receptor) family, pyrin domain-containing 3); autophagy linked NOD2, ATG16L1 (autophagy-related 16-like 1), IRGM (immunity-related GTPase) and DAP (death-associated protein); and epithelial barrier function linked GNA12 (guanine-nucleotide-binding protein α12), HNF4 (hepatocyte nuclear factor 4), CDH1 (cadherin 1) and LAMB1 (laminin β1). Autophagy was identified as a constitutive process regulating intracellular homeostasis and organelle regeneration in IBD. Mucosal immune tolerance was linked with bacterial persistence and the action of dendritic cells. Although many studies were unable to demonstrate consistent alterations in lymphocyte subsets, murine models provided independent support for a role of the T-cell response in IBD. Further studies identified IL-23 as a key cytokine in these events.

Heritability studies are progressing to address the problems of the low odds ratios observed and a possible role for epigenetics. Such studies are aimed at DNA methylation, histone acetylation, protein modification and microRNAs.

A significant aim of ongoing work is the determination of the factors which influence the site of disease and the behaviour/patterns of the disease. These fall into several categories, including genetic, bacterial, dietary, drugs, defensins and other antibacterial agents.

The gut flora is also a current focus of attention [1], but remains a complex field for study due to the large number of species identified in the gut (>1050 using 16S RNA typing experiments). Environmental factors have been found to play a significant role in IBD pathogenesis, but detailed identification is still limited. The assessment of risk alleles associated with host–microflora interactions and mucosal barrier protection has identified this as a fundamental target for future research. Examination of the gut-associated microflora, the gut microbiome, has attracted attention and is currently a major area of research. The links to inflammation are under study and have generated novel therapeutic options for IBD [7].

No conclusive data support the identification of specific pathogens in IBD. The design and preparation of probiotics for treatment of IBD is the crucial issue in effective action. The probiotic strategy in IBD requires evidence-based therapy, and the impact is likely to be moderate [8]. Much work on the use of pre- and pro-biotics has shown advantages in UC and pouchitis and has served to focus attention on the larger issue of the microbiota in mucosal protection.

IBD encompasses an abnormal immune response to the intestinal microflora coupled to chronic inflammation. Interaction and recognition of the intestinal microflora of gut cells of immune and non-immune origin is important in mucosal protection. Intestinal homeostasis may be disrupted through either increased or decreased innate immune signalling and indicates multiple roles for intestinal epithelial and white cell populations. These interactions lie behind the different disease mechanisms that have been proposed [9].

Normal individuals harbour high levels of T-cells in their intestine and this is triggered by the presence of the normal microbiota. Characteristically, IBD patients show abnormal activation of the T-cell population through currently poorly understood mechanisms and this results in the harm observed in the intestinal mucosa. In Crohn’s disease, T-cell responses appear to be due to the microflora. Pro-inflammatory cytokines play a role in this process, and the therapeutic use of TNFα (tumour necrosis factor α) has been effective. IL-21 and TGFβ1 (transforming growth factor β1) have been shown to have opposing functions. IL-21 is found to be pro-inflammatory and has an influence on T-cell survival and the maintenance of Th17 cells, whereas TGFβ1 is generally immunosuppressive [10].

Focus on the properties and functions of intestinal mucosal cells lining the gut has led to proposals regarding mechanisms of protection against xenobiotics and environmental bacteria. As part of the machinery dealing with these functions, the membrane-bound transporters such as the ABC (ATP-binding cassette) transporters and the anti-microbial proteins such as the defensins have been identified. Both of these groups of proteins are depleted in IBD. Deuring and co-workers [11] have examined possible mechanisms for these losses in relation to the correct folding of the proteins during biosynthesis. Loss of such folding generates the unfolded protein response and leads to ER (endoplasmic reticulum) stress and reduced functional capacity.

Acute intestinal inflammation mechanisms have been well studied and characterized. Mediators include prostaglandins, cytokines or reactive oxygen species, and leakage caused by epithelial injury together with changes in permeability. These processes are less well defined in chronic colitis, but include inhibition of secretory and absorptive processes and the role of ion transport. Sánchez de Medina and co-workers [12] offer a review of these changes, seen as a response to prolonged secretion. The mechanisms relate to the status of the transportome in chronic intestinal inflammation.

Current interest has been directed at oxidative stress as an aetiological factor leading to Crohn’s disease. The production of hydrogen peroxide by immune mononuclear cells occurs in Crohn’s disease patients. The antioxidant enzymes dealing with the peroxide have been identified. Although SOD (superoxide dismutase) activity and glutathione peroxidase levels are increased during active disease and return to basal levels in remission, catalase remains repressed. A potential role for catalase as a cell behaviour regulator is proposed by Iborra et al. [13] and discussed relative to reactive oxygen species, which may also regulate apoptosis.

Screening for IBD in children shows that the incidence of Crohn’s disease is increasing, whereas UC appears to have stabilized. There is also evidence showing that the use of antibiotics in early childhood can lead to the development of Crohn’s disease. In addition, current improvements in
household hygiene have been linked with the absence of environmental antigenic stimulus with no early adaptive immune response carried forward into adulthood and consequent increases in IBD susceptibility in later life.

Understanding of the impact of the diet on the gut microflora is vital to enable the stability and normal function of the gut [14]. It is clear that nutrition may have a role on a regular basis and has the potential to influence gut health and lead to disease. The metabolic responses found in different regions of the gastrointestinal tract vary. The microflora found in the colon is able to digest nutritional substrates that are not metabolized in the upper gut. The products of bacterial degradation are also relevant for the maintenance of the healthy gut. Molecular approaches to this situation have yielded valuable information defining the range and composition of the enteric gut flora. The establishment and pattern of the intestinal microbiome is age-dependent, with colonization starting at birth and maturing to characteristic adult composition over the first 2 years. Although inter-individual variation is significant, there is functional uniformity with regard to nutrition. The resident microflora is able to respond to nutritional disparity. The impact of the diet on gut health depends on the variety of specific bacterial communities and thus requires a strategy based on knowledge of an individual's microbiome composition.

An alternative diagnostic approach to IBD has evolved through the recognition of the characteristic odour of flatus or stool released during active disease [15]. Analysis of the gases, VOCs (volatile organic compounds), released is possible through trapping of the gas, a non-invasive procedure for the patients, and gas chromatographic/MS scanning (VOC profiling). Comparison of the profiles found for healthy subjects with patients with UC, Crohn's disease or infectious diarrhoea have yielded disease-related patterns. The compounds identified correlate with bacterial metabolism and reflect changes in bacterial populations in IBD and the host mucosal response. The body of data accumulating offers a novel non-invasive approach to detect and monitor gastrointestinal disease and IBD in particular.

The use of mouse models to probe the intricacies of IBD has proven to be a valuable tool. The Winnie mouse model of IBD, derived by Mike McGuckin and his group in Brisbane, Australia, has served to identify the link between inflammation and ER stress [16]. The development of IBD pathology is concomitant with changes to goblet cells and these are a hallmark for the disease. Fewer goblet cells are seen, the size of the thecae is reduced, there is vacuolization of the ER and Golgi apparatus and a reduction in the thickness of the mucus layer occurs.

The combination of data from the Winnie mouse model with human patient studies have identified the ER stress pathway as a major player in the development of intestinal inflammation [16]. The development of the inflammatory response in these mice can be traced to a mutation in the Muc2 mucin gene located in intestinal goblet cells. The misfolded Muc2 protein leads to ER stress and activation of the unfolded protein response. The development of inflammation as a result of a single gene polymorphism in secretory goblet cells is enabled through the Winnie model. The inflammation seen in these mice is progressive and most acute in the distal colon. A Th17-dominated inflammatory response is developed which also involves the innate immune system. Inhibition of tolerance in the Winnie mice aggravates colitis. The results implicate inflammation as a mediator of ER stress.

Similar patterns are found in other mouse models. Ingrid Renes and her group in Rotterdam have studied the Muc2-knockout mouse which shows increased host-bacterial interactions due to the loss of the mucus layer and increased PAMP (pathogen-associated molecular pattern) [LPS (lipopolysaccharide), LTA (lipoteichoic acid), CpG, DNA, peptidoglycan]-TLR2, TLR4 and TLR9 activation. In addition, inflammatory molecules, such as cytokines and chemokines, released in the submucosa play a role in modulating the mucosal response. Changes in body weight and epithelial cell proliferation are apparent after weaning. Increased Muc4 expression was found, and differences in the expression of the epithelial-specific innate defence molecule Reg3β (regenerating protein 3β) between proximal and distal colon were seen. Higher levels of Reg3β were found in proximal colon. More damage is seen in the distal colon of the Muc2-knockout mice at 4 weeks. A reduction in TLR2, TLR5 and My88 was seen with a corresponding decline in the TLR inhibitors Tipe2 and A20. In 4-week-old Muc2-knockout mice, the anti-inflammatory response declined, with fewer Treg cells (regulatory T-cells) and low IL-35 and TGFβ1 levels. Studies also demonstrated that diet can modulate the intestinal immune response in these mice. Different diets and probiotics showed influences on the influx of CD3+ cells and higher levels of Foxp3 (forkhead box P3), IL-12p35, TGFβ1 and Ebi3. The products of bacterial fermentation mediate these changes. Short-chain fatty acids and, in particular, butyrate at low concentrations (1–5 mM) modulated Muc2 mRNA expression. Thus dietary intervention can modulate intestinal gene expression, the intestinal inflammatory response and immunity.

Mouse models have also been adapted to examine other features of IBD. Vereecke et al. [17] report that A20 is an important regulator of the inflammatory response, acting through ubiquitination of members of the NF-κB (nuclear factor κB), IRF3 (interferon regulatory factor 3) and apoptosis signalling cascades. Mice with a full or conditional A20 deletion demonstrate its role in the prevention of chronic inflammation and autoimmune pathology. Polymorphisms within the A20 genomic locus are associated with a range of inflammatory and autoimmune disorders, including Crohn's disease. A20 is also thought to be a tumour suppressor in some B-cell lymphomas.

Animal models have demonstrated that histone deacetylase inhibitors may function in colitis to elevate levels of Foxp3+ Treg cells that dampen inflammation. Edwards and Pender [18] report that histone deacetylase inhibitors are capable of reversing the action of histone deacetylases, which
mediate the condensation of chromatin and inhibition of gene transcription. They allow the restoration of normal gene transcription and have been shown to act in an anti-inflammatory and anti-proliferative manner in cancer.

In summary, the Molecular Biology of Inflammatory Bowel Disease meeting provided a valuable review of current trends in research into IBD. The range of topics addressed serves to underline the broad basis necessary to understand the pathogenicity of these diseases. The meeting raised many questions which point to new areas and approaches for future work. The molecular nature of the data now available and the prospects for further developments at this level are encouraging and augur well for researchers in the field.

References


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