



INFLAMMATORY MEDIATORS IN IRRITABLE BOWEL SYNDROME

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Irritable bowel syndrome (IBS) is a chronic condition characterized by abdominal pain and altered bowel habit, in the absence of demonstrable structural gastrointestinal (GI) abnormality. Essentially, the gut functions abnormally -- people experience symptoms of pain, diarrhea and/or constipation, but no abnormality of the bowel can be identified by standard medical tests. Numerous research studies have now documented physiological changes, including altered motility and hypersensitivity, in the GI tract of IBS patients. The mechanisms underlying these events, however, remain unclear. There is growing evidence that inflammation in the GI mucosa may play a role in the pathogenesis of at least a sub-set of IBS(1-3). Importantly, overt colonic inflammation precludes a diagnosis of IBS. The challenge for researchers therefore, is to record subtle changes in inflammatory mediators that are not identifiable by usual routine processes.

INFLAMMATION AND IBS - THE EVIDENCE

Experimental (animal) data show that inflammation, even if mild, could lead to persistent changes in GI nerve and smooth muscle function, resulting in dysmotility, hypersensitivity and GI dysfunction(1, 4-6). In humans, the role of inflammation in the generation of IBS symptoms is less well studied, although there are important findings from people with 'post-infective IBS'. After an acute gastrointestinal infection, up to onethird (29%) of individuals develop persistent symptoms comparable to IBS(7, 8). In these post-infective IBS subjects, a persistent increase in rectal sensitivity(8) and in inflammatory cells (specifically enteroendocrine cells and lymphocytes)(9) have been documented. To put this in context, however, the majority (over 70%) of people who develop gastroenteritis fully recover and do not develop persistent bowel symptoms. Factors that may predict those who develop post-infective IBS include specific psychological factors, female gender and a longer duration of the initial illness. While there is now reasonable evidence to support a low-grade inflammatory response in those who develop IBS following an acute episode of gastroenteritis, there is still little know about inflammation in IBS patients who have no obvious recent evidence of acute infection. The role of inflammation in non post-infective IBS has been a particular research interest of our group and began with an interest in studying mast cells in IBS.

MAST CELLS AND IBS

The mast cell has potential to be a key player in IBS. Although mast cells are most widely known for their role in allergic responses, these cells are normally present throughout the gut and are involved in a range of physiological and pathological activities including mucosal defense mechanisms and inflammation. Mast cells

may release an array of inflammatory mediators that act on smooth muscle, nerves and immune cells, which may ultimately result in GI dysfunction and symptoms. In addition, mast cells in the gut are located close to nerves providing a structural basis for communication between the gut and the nervous system. In 1993, Weston and colleagues reported increased mast cell numbers in the mucosa of IBS patients(10) -- these changes were identified in tissue from the end of the small bowel (terminal ileum). Similarly, we identified significant increases in mast cells in IBS patients compared to controls in the colon (specifically at the caecum)(11). There were also trends for increases in other regions of the colon (ascending and descending colon), but no differences could be seen at the rectum. The lack of changes in mast cell infiltration in the rectum has since been confirmed by others(9). Collectively, these studies suggest that mast cells were increased in IBS patients -- this may be part of a low-grade inflammatory response, although the components and nature of this response remain poorly understood.

NITRIC OXIDE AND IBS

We hypothesized that inducible nitric oxide synthase may be a novel and important marker of inflammation in IBS. Enzymes from the nitric oxide synthase family are responsible for the synthesis of nitric oxide (NO) from the oxidation of L-arginine. While at least three enzyme sub-types (isoforms) have been described, our study focused on one specific isoform, namely inducible nitric oxide synthase (iNOS). This enzyme is thought to be capable of producing large amounts of nitric oxide and to be expressed during inflammation(12). In the human colon, up-regulation of iNOS has been implicated in inflammatory processes and increased expression has been documented in inflammatory bowel disease. In colonic mucosa from IBS patients, we found a significant increase in iNOS expression compared to control patients(13). We also measured nitrotyrosine, which is considered a by-product of nitric oxide generation. Overall, the nitrotyrosine data in IBS paralleled that of iNOS almost exactly. iNOS could be detected in epithelial cells, lymphocytes (CD3+ T and CD20+ B lymphocytes), and macrophages. Mast cells did not appear to express the iNOS. Overall, we demonstrated an increase in iNOS, an enzyme that catalyses the production of nitric oxide, combined with an increase in nitrotyrosine, a by-product of nitric oxide generation. In terms of factors that may affect iNOS expression, there was a trend towards higher iNOS levels in the diarrhea compared to the alternating group, although this was not statistically significant. Elevated iNOS may be a marker of general low-grade inflammation in IBS. Induction of iNOS and the generation of NO could have several other potential roles in IBS -- for example, NO may have direct effects on intestinal nerve and motor function and intestinal permeability.

OTHER INFLAMMATORY CELLS IN IBS

While there was some tentative evidence of an associated involvement of other inflammatory markers in our study, these were not striking or clear-cut. On average, there were trends for increases in CD3+ lymphocytes and the proportion of lymphoid tissue. Similarly, in IBS there was a trend for increased expression of nuclear factor kappa B, a transcription factor for an array of pro-inflammatory cytokines and mediators. In our study, none of these changes were statistically significant. In contrast, others have previously reported significant increases in inflammatory cells, for example an increase in CD3+ lymphocytes in post-infective IBS(9) and an increase in overall cellularity in the colonic mucosa in IBS(14). CD3 is a 'pan T-lymphocytes marker' and allowed us to estimate the overall number of T-lymphocytes in the lamina propria of the IBS patients. It is possible that specific subclasses of lymphocytes may be altered in IBS (e.g. CD4+ and CD8+ classes). Interestingly, the CD4+ T cell subtype has been implicated in an animal model of inflammation. In this model,

stress was shown to reactivate colonic inflammation (15) -- susceptibility to reactivation of inflammation by stress appeared to specifically require CD4+ lymphocytes.

ANTI-INFLAMMATORY MEDIATORS IN IBS

The study of inflammation in IBS has largely focused on the role of pro-inflammatory cells and mediators. From other gastrointestinal inflammatory diseases, we know that inflammatory responses involve a complex balance between pro- and anti-inflammatory mediators. There is emerging data to suggest that people with IBS may have a genetic predisposition to produce low amounts of anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β)(16). Moreover, there is a further preliminary report(17) of dysfunctional mucosal protective mechanisms in IBS characterize by reduced numbers of macrophages. While this data is provisional, it is plausible that people who develop IBS could have poorer defense mechanisms to combat infections or other gut insults.

A LINK BETWEEN INFLAMMATION AND STRESS

An increase in inflammatory mediators alone is insufficient to explain the pathogenesis of IBS. From post-infective IBS, we know that the most people who have gastroenteritis and accompanying inflammation do not develop IBS. As discussed earlier, several factors may be important in interacting with the gut insult and in predicting those who develop symptoms. One interesting factor here is the role of stress. Psychological stress is known to affect gut function in normal healthy humans and, in people with IBS, stress may trigger or exacerbate symptoms. More recently, there is evidence of a link between stress and inflammatory responses. Exposure to stress in both animals and humans has been shown to result in the release of mast cell mediators in the colon(18,19) and, in a recent study, stress was capable reactivating previous inflammation in rats(15). An important challenge for future work will be to clarify the interaction between inflammation, stress and IBS symptoms.

CONCLUSION

We believe that low-grade inflammation may play a role in the pathogenesis of a sub-set of IBS. There is now evidence from several different studies to suggest that a range of inflammatory mediators may be increased in IBS. It is important to point out, however, that most of this evidence, including our data, is preliminary and will need to be confirmed by larger research studies. Important challenges for future work will be to demonstrate the role of specific inflammatory mediators in the generation and resolution of IBS symptoms. Continued research into this area is likely to improve our understanding of the underlying mechanisms in IBS and may lead to the development to better therapeutic approaches for this condition.

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