Is HBx protein the X factor in the pathogenesis of IBD?
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The role of viruses in the aetiopathogenesis of inflammatory bowel disease (IBD) has been a topic of conjecture for decades. Initial focus on specific viral agents in IBD like norovirus, rotaviruses and measles virus did not gain ground. The advent of metagenomics and detailed assessment of the ‘whole gut virome’ has now shown that the gastrointestinal tract harbours nearly $10^9$ virus like particles per gram, with the majority comprised of prokaryotic viruses (bacteriophages infecting bacteria) and a minority of eukaryotic viruses.\(^1\) In IBD, the bacteriophages as apex predators in the gut ecosystem can alter their bacterial prey resulting in ‘dysbiosis’ of the bacterial population whereas eukaryotic viruses can interact directly with the host innate immune system and lead to chronic inflammation.\(^1\) This leaves us with the intriguing prospect that ‘viral dysbiosis’ could very well be the initiating event triggering the inflammatory cascade in IBD patients.\(^2\)

In this edition of Gut, Massimino et al have reported a novel association of viral infection and IBD.\(^3\) The authors have previously reported an upregulation of the HBx protein belonging to the Orthohepadnavirus genus, in the gut virome of early diagnosed, treatment naïve ‘paediatric’ subjects with ulcerative colitis (UC), but not in those with Crohn’s disease (CD) or normal subjects.\(^4\) In the current study, they confirmed HBx positivity in the biopsies of two cohorts of ‘adult’ UC patients ranging from 41-57%. Using effective screening, previous exposure or current infection with Hepatitis B, the only human virus of the Orthohepadnavirus genus was ruled out. Genomic HBx sequences were found in the blood of only 4% of these UC patients. This leads to the question as to how the HBx protein found its way to the gut mucosa, especially as most viruses in this genus are predominantly hepatotropic. Hepatitis B is known to persist in extrahepatic tissues like the bone marrow, without systemic evidence of disease and it is likely that trafficking of infected cells could lead to its presence in the gut.\(^5\) However, the HBx transcript was detected in both immune and non-immune cell populations.
isolated from HBx+ UC patient-derived colonic mucosae and this question still remains unanswered. The other alternative is that there are other hitherto unknown members of the *Orthohepadnavirus* genus, possibly zoonotic species of viruses, which are enterally acquired through contaminated food and water leading to chronic exposure of the gut.

The authors reported a set of compelling studies to highlight the putative role of this protein in the aetiopathogenesis of UC. Transcriptomic analysis of healthy donor-derived mucosal biopsies transduced with lentiviruses carrying the HBx-IRES-GFP encoding sequence showed an upregulation of genes pertaining to microbial response (e.g., TLR signalling) and pro-inflammatory pathways (e.g., TNFα and interleukins) and down regulation of those involved with viral clearance (e.g., interferon). This pattern is similar to that seen in liver tissues in chronic hepatitis B, where ongoing viral replication and ineffective inflammation go hand in hand. This was further borne out by the *in vivo* experiments wherein mice exposed to the HBx protein developed colitis with an altered immune milieu where dendritic cells, CD8+ T cells, and neutrophils were reduced in number. Dendritic cells are the bridge between innate and adaptive immunity and their dysfunction has been similarly documented in chronic hepatitis B, drawing a further parallel between these two scenarios.6 The importance of the HBx protein was borne out by reversal of the inflammation on treatment with HBx-targeting siRNAs. This pro-inflammatory effect of the HBx protein was independent of the gut bacteria as pretreatment of the mice with two weeks of an antibiotic cocktail did not reverse the colitis. The authors finally demonstrated that HBx-overexpressing Caco-2 cells had an increased proliferation rate and a reduced trans-epithelial electrical resistance (TEER) indicating that it resulted in intestinal barrier dysfunction as well.

The HBx protein is a small protein of 154 amino acids encoded by the open reading frame-X (X-ORF) of the HBV genome, which is highly conserved within the *Hepadnavirus* family.7 Its importance in hepatocarcinogenesis was initially highlighted by the fact that avian hepadnaviruses infection, where X-ORF is absent, is not associated with liver cancers. HBx protein appears to have myriad effects that include several cellular functions such as
cytoplasmic calcium regulation, cell signalling, transcription, cell proliferation, DNA repair, and apoptosis which are all implicated in some measure in its oncogenic potential. It exerts these effects by binding the host DNA and acting as a transcription regulator. Massimino et al have demonstrated a similar effect in intestinal cells and it may be speculative that a similar effect driven by the HBx protein maybe be related to colorectal carcinogenesis in patients with UC. Further studies are needed in UC patients who develop colorectal cancer to see if this protein is preferentially upregulated in their mucosa. The HBx protein could then be an important predictor for carcinogenesis and a potential target for preventing cancer in UC patients.

The final rhetorical question that comes to mind is why this protein was restricted to the mucosa of patients with UC and not CD. It is quite simplistic to divide CD and UC on the basis of the effector T helper subsets Th1 and Th2 respectively, especially with the discovery of the unique subset of IL-17-producing Th17 cells. It is important that the authors found the HBx protein in both paediatric and adult patients with ulcerative colitis highlighting its importance as an initial trigger for inflammation and not an epiphenomenon in later years due to repeated flares of inflammation and immunomodulatory treatment. It is increasingly being recognized that asymptomatic hosts carrying viruses may have a persisting immune response that leads to a ‘continuum’ of background inflammation which makes them susceptible to other diseases. Drawing the parallel with Hepatitis B again, the ‘chronic carrier’ state is a deceptive state of harmony between the host and the virus but with the residual risks of flares of inflammation and cancer always present in the background. Chronic viral infections invariably try to switch the effector T helper subsets from Th1 to Th2 in order to survive, and in the gut, it may inadvertently lay the groundwork to develop ulcerative colitis. The HBx protein may indeed be the X factor that we are looking for in the pathogenesis of IBD and a potential new therapeutic target.
References


