Inflammatory bowel disease

Immune regulation and colitis: suppression of acute inflammation allows the development of chronic inflammatory bowel disease

B Eksteen, L S K Walker, D H Adams

Persistent colitis is the result of a balance between local inflammation and regulatory networks. Regulatory T cells have potent anti-inflammatory effects and are likely to be important in the pathogenesis of chronic inflammatory bowel disease

The success of the gastrointestinal immune system depends on a balance between mounting effective immune responses to pathogenic antigens while suppressing potentially damaging responses against commensal organisms or food antigens. Both the innate and acquired immune system contribute to fighting pathogens. The innate immune system, which includes phagocytes, dendritic cells (DCs), and natural killer (NK) cells, does not require previous exposure to a pathogen and instead relies on evolutionarily ancient pathways such as Toll-like receptors (TLRs) to recognise molecular patterns associated with harmful pathogens.[1] Different TLRs are able to recognise bacterial products or motifs in viral RNA or DNA. TLR activation triggers an immediate response resulting in the activation of phagocytic mechanisms and the production of cytokines and costimulatory signals that activate the cognate immune response.

The cognate or acquired immune system developed in higher vertebrates to provide a more sophisticated response to a wide variety of antigens. It involves lymphocytes that recognise specific antigens from pathogens processed and presented by specialised antigen presenting cells called DCs. A crucial feature of the cognate immune system is immunological memory whereby a subsequent exposure to the same antigen leads to a more potent and sustained immune response. Antigens from pathogens that penetrate the mucosal barrier are taken up by local DCs and carried via lymphatics to draining mesenteric lymph nodes.[2] In addition, DCs situated in gut associated lymphoid tissue such as Peyer's patches sample luminal antigens either directly, by extending finger-like processes into the lumen, or via specialised epithelial cells (M cells) that actively transport luminal antigens to the underlying DCs.[3] Antigen containing DCs then migrate to secondary lymphoid tissues where they interact with naïve lymphocytes with the appropriate antigenic specificity to generate primed effector lymphocytes expressing the receptors CCR9 and $\alpha4\beta7$ that direct their migration back to gut tissues.[4–6] Once the antigen is cleared, most effector cells die, leaving a small cohort of long lived memory cells that can rapidly augment immunity to the antigen if encountered again.

The importance of our ability to develop immunity and appropriate responses to gut derived antigens is highlighted by, on one hand, immunodeficient states where persistent gut infections often dominate and translocation of gut pathogens can lead to overwhelming sepsis, and on the other, the fact that inappropriate activation of the intestinal immune system leads to uncontrolled inflammation and inflammatory bowel disease (IBD).[7] Recent studies suggest that damaging inflammation in IBD is not only the consequence of inappropriate stimulation of effector responses but is also due to a failure of the normal immunosuppressive mechanisms that have evolved to control inflammation in the gut.

The immune system has developed several ways to suppress responses to harmless self or non-pathogenic environmental antigens. Central tolerance is regulated in the thymus where T cells are selected for survival based on the affinity of their T cell receptor (TCR) for antigens expressed on thymic epithelium and those T cells showing high affinity for self antigens are deleted. In addition, thymic selection leads to generation of a distinct subset of regulatory T cells (T_{regs}) which when activated by antigen can suppress effector responses generated by both NK and T cells in the periphery.[8] Thymic T_{regs}, which comprise approximately 10% of CD4+ T cells in the mouse circulation, display several cell surface receptors, including the interleukin (IL)-2 receptor, CD25, and the glucocorticoid induced tumour necrosis factor (TNF) receptor (GITR). They are characterised by expression of the transcription factor Foxp3 which is

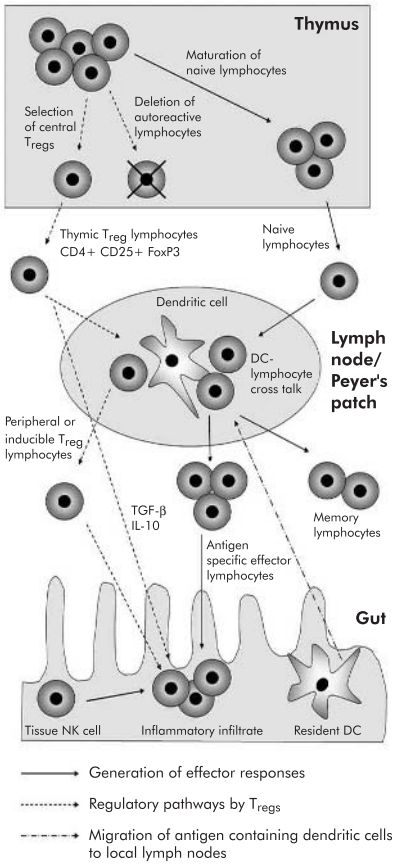

Figure 1 Simplified pathways involved in the generation of effector and regulatory lymphocyte responses. Naïve lymphocytes that have been selected and matured in the thymus are able to enter secondary lymphoid tissue such as lymph nodes and Peyer's patches where they interact with dendritic cells (DC) that have assimilated antigens in the gut. Interactions with DCs subsequently prime the naïve lymphocytes to the antigen and activate their differentiation into effector lymphocytes. Part of this differentiation is induction of tissue specific homing molecules that direct the effector cells back to the tissue where the antigen was found. During this process memory lymphocytes are also generated to allow for rapid expansion of effector cells if the specific antigen is encountered again. Regulation occurs at several levels with autoreactive lymphocytes being deleted in the thymus and generation of central/ thymic regulatory T cells (T_{regs}). Peripheral or induced T_{regs} are also generated locally by DC-lymphocyte interactions. Both sets of T_{regs} are able to control inflammation by the production of anti-inflammatory cytokines such as transforming growth factor β (TGF-β) and interleukin 10 (IL-10).

critical for T_{reg} function, as demonstrated by experiments in which retroviral transfer of Foxp3 to naïve T cells converts them into functional T_{regs} whereas its deletion results in loss of regulatory function and the development of autoimmunity.[7 9 10] T_{regs} mediate contact dependent suppression of effector cells in vitro, although in vivo the situation is more complex with IL-10 and/or transforming growth factor β (TGF-β) being required for suppression in many circumstances. Indeed, it has been reported that T_{regs} express a membrane bound form of the TGF-β cytokine.[11] In addition to thymic T_{regs}, inducible T_{regs} can be generated in the periphery as a consequence of activation of naïve T cells by immature DCs or activation in the presence of specific cytokines. Thus generation of inducible T_{regs} depends critically on the local microenvironment in which activation takes place. Inducible T_{regs} secrete IL-10 and stimulate the local secretion of TGF-β, both of which are potent regulators of inflammation capable of suppressing the proliferation of effector cells.[8 12]

Powrie *et al* originally defined the importance of T_{regs} in gut inflammation, demonstrating that adoptively transferred T_{regs} suppress colonic inflammation in experimental colitis and that this suppression depends on both TGF-β[13] and IL-10.[14] The subsequent demonstration that IL-10 can promote TGF-β secretion in the setting of experimental colitis provides a further clue as to how these cytokines may be cooperating in vivo.[15] The importance of IL-10 is underscored by the fact that deficiency in either IL-10[16] or in the ability of macrophages/neutrophils to respond to IL-10 (as a result of targeted stat-3 deficiency)[17] is sufficient to trigger gut pathology. Likewise, removal or inhibition of T_{regs} at either the central or peripheral level is associated with autoimmunity,[18] a break in tolerance, and intestinal inflammation in animal models.

Much of the decision making that leads to generation of peripheral regulatory networks falls upon DCs and suppression and tolerance are the consequences of lymphocyte activation by immature DCs that produce IL-10.[19 20] Further control of inflammation in the periphery is afforded by the susceptibility of effector lymphocytes to programmed cell death or apoptosis.[18] Mechanisms that have evolved to control the expansion of antigen specific effector cells are critical for normal immune homeostasis to prevent the uncontrolled proliferation of effector cells. IL-2 is a survival signal for activated lymphocytes which is produced in large amounts during inflammation. When the antigen has been cleared, IL-2 production falls, resulting in apoptosis of most effector cells except for a small population of long lived memory lymphocytes.[21] In addition, effector lymphocytes express the death receptor Fas and its ligand Fas-L and once cytolytic T cells have destroyed Fas bearing target cells, effector T cells can kill each other by the same Fas/FasL interactions allowing inflammation to resolve. Curiously, IL-2 potentiates Fas mediated killing, illustrating the complex interplay that exists between different peripheral tolerance mechanisms. The importance of the Fas pathway is demonstrated by the development of lymphomas in mice that lack either Fas or FasL.

Mechanisms that lead to uncontrolled chronic inflammation in IBD are poorly understood but are likely to involve many if not most of the above mechanisms.[22] The paper by Westendorf and colleagues[23] in this issue of *Gut*, provides further important insights into the mechanisms of chronic inflammation in IBD ***(see page 60)***. The authors used a transgenic model in which gut inflammation was triggered by overexpression of a single foreign antigen, influenza HA, in enterocytes. Crossing these mice with transgenic animals that expressed αβ-T cells specific for HA resulted in an animal with autoreactive T cells that recognised HA as a ''self'' antigen restricted to the gut. They reported that these animals developed autoimmune colitis and chronic inflammation demonstrating that expression of a self antigen on enterocytes in sufficient to trigger colitis. However, they found that although their model induced chronic inflammation, colitis was far less severe than in other colitis models, suggesting that the inflammation is partially controlled by regulatory mechanisms. The authors then studied the cytokine profiles secreted by mucosal lymphocytes from their transgenic animals in response to antigen stimulation. They found that whereas secretion of the classical Th1 effector cytokines interferon γ and IL-2 was reduced, secretion of the proinflammatory cytokines TNF-α, monocyte chemoattractant protein 1, and IL-6 was increased, indicating that while T cells were capable of responding to antigen, the nature of the response was markedly altered. This raises the possibility that a balance has developed between regulatory and inflammatory mechanisms giving rise to the generation of chronic persistent inflammation. When the authors pursued this hypothesis further they found that autoreactive lamina propria lymphocytes and intraepithelial lymphocytes expressed increased levels of the anti-inflammatory cytokine IL-10 and several genes associated with the development of regulatory T cells. While some of these genes would also be highly expressed in activated T cells (for example, OX40, GITR), others such as neuropilin-1 are thought to specifically identify regulatory cells.[24] The authors concluded that, in their double transgenic mouse, enterocyte specific CD4+ T cells are sufficient to induce colitis which is neither acute and self limiting (which might be expected if regulatory mechanisms were dominant) nor acute and fatal (if they were absent) but rather chronic and persistent. They propose that this outcome is the result of a balance between local inflammatory and regulatory networks and infer that regulatory T cells may be important in the pathogenesis of chronic inflammation.

Several important conclusions regarding the pathogenesis of IBD can be drawn from the study and there are parallels between the VILLIN-HA×TCR-HA transgenic model of gut inflammation and IBD in humans. Recognition of an autoantigen by either breakdown of central tolerance in the thymus or by acquired cross reactivity to an external antigen provides a potent immune response that is able to establish clinical disease. However, the pattern of disease may well be determined by the nature of the local tolerogenic networks. This is turn will be affected by (1) the genetic background of the individual, which will determine whether they generate strong regulatory or inflammatory responses, and (2) the local microenvironment, including the nature of the bacterial flora. The paper demonstrates the importance of effective thymic selection in deleting autoreactive T cells. This is not a new concept—loss of thymic regulation has been shown to trigger a variety of autoimmune conditions, including colitis—but is nevertheless important as these animals had no obvious defects in the thymus. The authors concede that the transgenic TCR might interfere with thymic selection but it is also plausible that the large numbers of potentially autoreactive lymphocytes in this transgenic model allow some to escape deletion. It is not known if this phenomenon occurs in humans with IBD.

A second important finding is the pattern of inflammation induced in the VILLIN-HA×TCR-HA mice. Models of colitis induced by agents such as dextran sulphate result in acute inflammation and require repeated administration to mimic chronic gut inflammation whereas the authors' model of autoimmune intestinal inflammation is sustained by the persistent presence of the gut antigen. However,

the real novelty of the present study is the suggestion that the chronicity of the inflammation, which in many ways resembles clinical IBD, is the result of a balance between pro and anti-inflammatory pathways involving regulatory T cells. This balance permits continuing inflammation while preventing uncontrolled progression of destructive colitis. Factors underlying this regulatory response are at present unknown although the data in the paper suggest testable theories. The cytokine data suggest that local IL-10 is able to prevent acute destruction of the gut but not chronic intestinal inflammation, leading to persistence of inflammation and chronic disease. Local intestinal DCs are a potential source of IL-10 and may induce the development of IL-10 secreting T cells. Because the transgenic model expresses local antigen in the absence of an inflammatory ''danger'' signal, local DCs may be only partially activated, leading to generation of immune responses dominated by IL-10 secretion. The gene expression data suggest that regulatory T cells are involved, and the nature, localisation, and function of these cells will be important to determine. For instance, are these thymic T_{regs} or T_{regs} induced locally by high levels of IL-10 and immature DCs? Is this regulatory network driven by persistent antigen and what is the role if any of gut epithelial cells in maintaining local T_{regs}? The concept that regulatory T cells are required for the development of chronic inflammation is intriguing and suggests that these cells are more than simple anti-inflammatory agents. The signals that lead to the development and persistence of chronic inflammation are poorly understood and the involvement of regulatory T cells adds another element to the pathogenesis of chronic inflammatory disease (fig 1).

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Authors' affiliations
B Eksteen, L S K Walker, D H Adams, Liver Research Laboratories and MRC Centre for Immune Regulation, Institute for Biomedical Research, Queen Elizabeth Hospital, University of Birmingham, Birmingham, UK

Correspondence to: Professor D H Adams, Liver Research Laboratories, Institute for Biomedical Research, Queen Elizabeth Hospital, University of Birmingham, Birmingham B15 2TT, UK; d.h.adams@bham.ac.uk

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Crohn's disease

Will worms really cure Crohn's disease?

G L Radford-Smith

Treatment of Crohn's disease patients with the intestinal helminth *Trichuris suis* appears safe and effective in the short term, even with concurrent immunosuppressive therapy

HELMINTHS AND IBD EPIDEMIOLOGY

There are a wealth of data that support an immunoregulatory role for helminth infection in animal models and the human host.[1–3] Recently, this concept has been utilised therapeutically for the treatment of patients with inflammatory bowel disease (IBD). Specifically, Summers and colleagues[4] report the results of their open study of live *Trichuris suis* ova therapy in 29 patients with Crohn's disease (CD) in this issue of *Gut* ***(see page 87)***.[4] Treatment with *T suis* appears safe and effective in the short term, even with concurrent immunosuppressive therapy. Extension of this concept into the ''hygiene hypothesis''[5] may seem increasingly attractive in terms of an explanation for some epidemiological observations in patients with IBD, in particular the north-south gradient for IBD prevalence in both North America and Europe, and the lack of IBD in developing nations.[6–8] However, some of these epidemiological observations should be viewed with caution. Studies

that find a north-south gradient are limited to determining incidence rates and do not attempt to tackle the more difficult task of finding epidemiological reasons behind the gradient. In addition, the stringent "rules" that are now being applied to replication of genetic association and linkage studies have not been applied to all epidemiological studies in IBD. Recent data on paediatric IBD and twin studies would support an increasingly important role for environmental factors over genetics. Specifically, paediatric IBD has increased in frequency in the recent past (1990–2001), is becoming increasingly multi-ethnic, and less often familial, and repeat twin studies from Scandinavia using a new (younger) cohort show reduced concordance rates for CD.[9–11] Multiple environmental factors, including pathogen exposure, diet, and lifestyle, are likely to contribute to these observations. However, the role of helminth infection is questionable here given the age of these cohorts, their geography, and the switch from ulcerative colitis (UC) to CD as the leading cause of IBD seen in at least two studies.[9 12]

IS INTESTINAL IMMUNOREGULATION THE EXPLANATION?

Chronic helminth infection affects over one billion people worldwide, and although these individuals may suffer subsequent nutritional and growth deficiencies, they rarely develop allergic or chronic autoimmune disease.[5] Immunologically, this is thought to relate to two major processes. Firstly, helminthic infection is associated with a strong Th2 response, which opposes the Th1 response associated with autoimmune disease and CD.[1–3] Secondly, chronic infection with these organisms may generate a network of regulatory T (Treg) cells that secrete transforming growth factor (TGF)-β and interleukin (IL)-10.[2 13] These cytokines may not only regulate aggressive Th1 responses but also control heightened Th2 responses that contribute to chronic allergic diseases. The data supporting these pathways come from both human and animal studies, with IL-10 levels elevated in chronic schistosome infection and reduced in patients with chronic allergic diseases from industrialised countries.[14 15] However, there are limited data to confirm the role of these pathways in the human gut. Animal models of IBD such as the trinitrobenzene sulphonic acid colitis murine model have indicated that resolution of inflammation in animals infected with *S mansoni* eggs is associated with increased mucosal IL-10 and reduced interferon-γ (IFN-γ) mRNA, while IL-4 levels are increased in the mesenteric lymph nodes.[16] These results are in contrast to those of Moreels *et al* who used the same model to demonstrate significant attenuation of colitis in animals with helminth infection, but were unable to confirm the Th1 to Th2 switch.[17] Data presented recently in abstract form by Elliott *et al* indicate that helminth infection (*H polygyrus*) in the proximal small bowel is able to influence immunoregulatory cytokines downstream in the Peyer's patches of the terminal ileum. Infection is associated with downregulation of IFN-γ, upregulation of IL-4, IL-5, and IL-10, and a switch in lipopolysaccharide induced cytokine synthesis, from IL-12 to TGF-β[18 19] All of these experiments have the limitations of being carried out in the highly controlled environment of animal models, and some also involve a cell isolation step. No similar human data are available. However, experience has taught us that human IBD is not as simple as Th1 versus Th2,[20] and therefore other "anticolitis" mechanisms of protection and repair may be in place during helminth infection. It is not so long ago that the concept of a breakdown in oral tolerance was put forward as a potential mechanism for human chronic inflammatory disorders, including IBD. This hypothesis has a very similar cast of cytokines, including IFN-γ, IL-2, IL-4, IL-10, and TGF-β. Successful trials of promoting oral tolerance in animal models are yet to realise their potential in patients.[21]

OTHER PROTECTIVE MECHANISMS

Helminth infection may bring other anticolitis mechanisms into play, including increases in mucus and water secretion into the gut lumen via effects on goblet cells and mast cell activation.[22 23] This may influence the interaction between gut bacteria, their products, and a diseased epithelium, as well as impacting on intestinal motility. Helminths may also influence the microbial ecology of the gut[24] and the neuroendocrine response, with an increase in neurotransmitters such as vasoactive intestinal polypeptide.[25] None of these factors has been assessed in human studies.

WIDENING THE CONCEPT OF PATHOGEN EXPOSURE IN IBD

There are other "pathogens" that may give us clues to a more complex interplay between the environment and the human host in IBD. A number of studies show a reciprocal relationship between exposure to *Helicobacter pylori* and IBD, particularly CD.[26 27] At least two studies have gone on to demonstrate that *H pylori* exposure may be associated with subtle changes in disease course, including a delay in CD presentation[28] and a reduction in relapses in non-smokers with CD,[29] despite the known mucosal Th1 response associated with *H pylori* infection.[30] These studies have thus far been relatively small and some of the results may be due to a cohort effect. However, the results are consistent with increased levels of domestic hygiene in CD patients, and *H pylori* in this situation may be a surrogate marker of the spectrum of environmental exposures in infancy and childhood. Similar exposure data are not available for helminth infection in the IBD population. Data are available for allergic disease and exposure to *Enterobius vermicularis* within a similar "developed world" population, and do not show any protective effect of infection over allergy.[31] However, "infection turnover", as opposed to specific infections, may also play an important role in the development of a balanced immune system by generation of increased regulatory T cells. An example of this may be childhood/adolescent appendicitis and its potentially protective role against the development of UC.[32] There are two further pieces of recent evidence that indirectly support the concept of reduced pathogen exposure in childhood as a risk factor for IBD. Baron *et al* identified breast feeding as being an independent risk factor for the development of paediatric CD.[33] This may work in a number of ways, one of which is to provide the infant with blocking antibody to an array of dietary and microbial antigens and thus reduce both oral tolerance and pathogen exposure at a critical stage of immunological development. Secondly, analysis of the mucosa associated bacteria of patients with active IBD and controls suggests that patients have a reduction in the diversity of intestinal bacteria compared with the control group.[34] However, this may be confounded by a greater uptake of antibiotics in patients with CD, as recently reported in *Gut*.[35] Both of these studies[33 34] need to be replicated by independent investigators. These observations together with the results of helminth infection suggest that the microbial environment of the entire gut may have an influence on the development of intestinal inflammation. Further work on the epidemiology of these early exposures and the microbial ecology of the whole gut are essential in identifying key environmental risk and protective factors for IBD.

IS THERAPEUTIC HELMINTH INFECTION SAFE?

Complications related to therapeutic helminth infection have not arisen thus

far. However, there is evidence that coinfection with other known pathogens such as *Campylobacter jejuni* may result in serious infection, including septicaemia.[36] A recent case report indicates that this coinfection and its serious consequences may also occur in patients.[37] Another potentially serious coinfection is *S mansoni* with *Toxoplasma gondii*, which leads to a significant increase in circulating tumour necrosis factor α, severe liver pathology, and death in a murine model.[38] These reports indicate that patients being considered for helminth therapy may require screening for carriage of other potential pathogens prior to initiation of treatment. Other issues related to this that need to be addressed by future studies are the choice of organism and the type of infection. Both human and animal studies indicate that a heavy helminth burden is associated with a greater immunoregulatory environment, while a light burden may be associated with an increased risk of allergic disease.[5] The current human trials in IBD patients use a transient infection and, despite this, demonstrate clinical efficacy and no significant allergic disease post-infection.[4] Long term data, particularly after repeated exposure, will provide further reassurance.

SUMMARY

In conclusion, helminth infection provides us with an excellent model of a successful parasite which is able to manipulate its environment within the host to its advantage. Summers and colleagues[4] have now taken that further by using helminths to gain an advantage for the host. It is too early to determine whether this form of treatment will be safe and effective for larger numbers of patients with IBD—further controlled randomised studies will be required to answer this. However, what these important and innovative studies demonstrate is the need for a greater understanding of the helminth-host relationship. This is slowly being addressed but almost exclusively in animal models. Of special interest will be identification of antigens or epitopes responsible for the generation of a tolerant environment, and recent work indicates that one candidate is the schistosome oligosaccharide lacto-N-neotetraose.[39] This molecule, which is also present in human milk, stimulates the expansion of a $Gr1^+$ cell population, which creates a Th2 biased immune environment by increased production of IL-10 and TGF-β, and by directing naïve $CD4^+$ T cells down the Th2 path. Molecules such as this may represent potentially novel therapeutic agents for chronic inflammatory disorders such as IBD, and thus bypass the need for helminth inoculation and infection.

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Correspondence to: Dr G Radford-Smith, Department of Gastroenterology, Level 9A, Ned Hanlon Building, Royal Brisbane and Women's Hospital, Herston 4029, Australia; graham_radford-smith@health.qld.gov.au

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Lamivudine therapy

Resistance to lamivudine therapy: is there more than meets the eye?

G Dusheiko, A Bertoletti

A CD8+ T cell response to lamivudine resistant polymerase cytotoxic T lymphocyte (CTL) epitope influences the response to lamivudine treatment for chronic hepatitis B and may indicate an important aspect of the role of T cell responsiveness in lamivudine therapy

The goals of treatment of hepatitis B are to prevent progression of the disease or to slow the disease process. Hepatitis B virus (HBV) is a DNA virus which integrates into the host genome. Thus it is difficult to eradicate viraemia. However, it is possible, albeit in a minority, to reduce levels of viraemia to relatively low threshold levels after finite courses of treatment with either interferon alpha or nucleoside analogues, and to lessen the induced necroinflammatory and immune response.[1] Two major forms of active chronic hepatitis B are recognised: wild-type (or hepatitis B e antigen (HBeAg) positive chronic HBV infection) and anti-HBe positive or precore mutant disease. The latter disease is caused by variants of HBV that contain nucleotide substitutions in the core promoter/precore regions of the viral genome.[2]

Wild-type (HBeAg positive) chronic hepatitis B can be treated with either interferon alpha or nucleoside analogues. Loss of HBeAg and associated suppression of viral replication with pegylated interferon alpha and new nucleoside analogues such as lamivudine, adefovir, tenofovir, telbuvidine, entecavir, and emtricitabine leads to biochemical remission, histological improvement, and in a small percentage, loss of HBsAg.[3–5] Durable responses can occur. Continuous therapy is frequently required for the majority of anti-HBe positive patients with chronic hepatitis B. Thus in most patients, longer term therapy is required to suppress viral replication and thereby slow the disease process.

Therapy with lamivudine alone to suppress viral replication, leading to normalisation of alanine aminotransferase (ALT) levels and improvements in liver histology, unfortunately leads to the emergence of lamivudine resistant mutants in over 50% of treated patients within three years of treatment.[6 7] The immediate clinical impact of resistance is usually relatively minor, although ALT flares and hepatic decompensation can occur.[8] Earlier short term studies suggested that resistant HBV mutants were less fit (that is, were less replication competent) and consequently less pathogenic. Subsequent studies have indicated that incomplete suppression of HBV replication reduces the degree of benefit. Drug resistant virus favours the selection of increasingly fit and equally pathogenic virus by viral adaptation, and given the complex immune response to hepatitis B, such variants are indeed pathogenic over time.[9–12] Resistance to lamivudine results in lower seroconversion rates in HBeAg positive patients, lower rates of virological and biochemical remission, and less favourable histological change and thus an adverse effect on treatment outcome.[13]

Lamivudine resistance has been mapped to mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif of the reverse transcriptase (rt) domain of HBV DNA polymerase.[14] Methionine 204 is mutated to isoleucine or valine (rtM204I/V) in patients with increasing viraemia. Although predictors of resistance have been incompletely defined, immunosuppressant therapy, precore mutants or core promoter variants, duration of therapy, higher baseline ALT, higher baseline HBV DNA, body mass index, and HBV subtype adw have all been implicated.[15] Mutational patterns may also differs between genotypes A and D.[16]

Lamivudine treatment have been shown to enhance CD4 and CD8-T cell response to HBV antigens.[17 18] In this issue of *Gut*, Lin and colleagues[19] suggest that a CD8+ T cell response to lamivudine resistant polymerase epitope influences the response to lamivudine ***(see page 152)***. They examined the function and phenotype of specific T cells and demonstrated that functional anti-YMDD cytotoxic T lymphocytes (CTL) correlated with response and outcome. The authors capitalised on the fact that the YMDD motif of the rt domain of HBV DNA polymerase within the nonapeptide YMDDVVLGA (amino acid residues 203–211) in the catalytic site of the HBV DNA polymerase, is an HLA-A2-restricted CTL epitope.[20] Thus quantitative measurement of the numbers of peptide specific CTLs is feasible by MHC tetramer-peptide complex staining. The peptides used for analysis included the dominant HLA-A2 restricted peptide from the HBcAg18–27 (FLPSDFFPSV), the wild-type YMDD motif nonapeptide (YMDDVVLGA), the mutant YVDD peptides (YVDDVVLGA), and YIDD peptide (YIDDVVLGA), to construct HLA-A2-peptide tetrameric complexes.

The authors demonstrated that the frequency of YMDD, YIDD, and YVDD motif specific tetramer positive cells within the HLA-A2 CD8 T cell population was increased in ''sustained responders'' (that is, those with clearance of HBeAg and sustained normalisation of ALT after the end of lamivudine therapy) but not in ''non-responders''. The interesting scenario proposed by the authors is that these anti-mutant CTLs may also contribute to clearance of emerging mutant viruses and a successful response to lamivudine treatment. The implication is that treatment responses are improved (and conversely rates of resistance reduced) if CD8+ T cells respond to treatment and proliferate, resulting in a higher level of functional anti-mutant CTL activity during and after therapy.

Some aspects however need to be considered. Firstly, the HBV specific CD8+ T cells were detected in this study only after repetitive in vitro expansion, a technique that detects a small number of precursors. Thus the frequency of polymerase specific CD8+ cells was lower than frequencies usually detected in patients controlling HBV infection.[21] In addition, the in vivo antiviral efficacy of polymerase specific CD8+ T cells is under debate. The ability of polymerase specific CD8+ cells to control viral replication seems absent in HBV transgenic mice[22] and in patients with chronic hepatitis B.[21] Thus the increased presence of YMDD, YIDD, and YVDD specific CD8+ cells might be merely an association, and not the primary basis of the sustained response to lamivudine treatment. Other CD8+ T cells, not necessarily polymerase specific, could be present in sustained responders to control viral replication.

The ability of YMDD specific CTLs to cross react with YIDD and YVDD mutant epitopes is also not totally unexpected.

As the authors point out in their discussion, the amino acid substitution in the YMDD epitope is located at position 204, which corresponds to the anchor position of the epitope and does not affect T cell recognition. Thus demonstration that YMDD specific CTLs can be activated by YIDD and YVDD mutant epitopes shows that the isoleucine and valine mutations at position 204 do not abrogate the binding of the epitope to HLA-A2, and that recognition of lamivudine resistant mutant epitopes does not require induction of a new cross reactive CTL response. However, it must be considered that these mutations might have an impact on the immunogenicity of the YMDD region. It will be important, for example, to directly test whether these YIDD or YVDD lamivudine resistant epitopes bind with higher affinity than YMDD to HLA-A2 molecules. Improved binding of the mutant peptide to HLA-class I molecules would increase the presentation of the lamivudine resistant epitope to specific CTL. This could potentially enhance the ability of polymerase specific CD8+ cells to recognise HBV infected cells in vivo and thus explain the association of polymerase specific CD8 and sustained viral control during lamivudine treatment in HLA-A2+ patients.

Demonstration that polyclonal activation of anti-wild type and CTL specific immune responses to YMDD, YIDD, and YVDD epitopes correlates with treatment outcome is an intriguing and new finding, and may point to an important aspect of the role of T cell responsiveness in lamivudine therapy. If confirmed, these new findings may assist in devising a strategy for treatment of patients and recognition of patterns of genotypic and phenotypic resistance. Lamivudine is a useful treatment for chronic hepatitis B, but its usefulness as a single therapy is limited by the frequency with which resistance occurs. It will be important to ascertain how treatment outcomes can be improved by further insights into immune responsiveness during antiviral therapy.

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Authors' affiliations
G Dusheiko, A Bertoletti, Centre for Hepatology and Institute for Hepatology, Royal Free and University College School of Medicine, London, UK

Correspondence to: Professor G Dusheiko, Centre for Hepatology and Institute for Hepatology, Royal Free and University College School of Medicine, Pond St, London HA5 1DE, UK; g.dusheiko@rfc.ucl.ac.uk

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