compromise the correct interpretation of these morphogenetic signals by mutating their receptors or intermediate signalling molecules. This may enable clonal growth of cancerous cells by helping them to escape the normal position dependent growth restraints that morphogenetic pathways impose. The classic examples are mutations in the Wnt pathway. The constitutive activity of Wnt signalling that results from these mutations can be inhibited by Hh signalling in colon cancer cells. Akiyoshi *et al* now provide the first evidence for an antagonistic interaction between the Hh and Wnt pathways in colorectal cancers in vivo and show that this interaction probably occurs at a downstream level in both pathways.

Gut 2006;**55**:912–914.

doi: 10.1136/gut.2005.085902

.....

Authors' affiliations

G R van den Brink, Department of Experimental Internal Medicine, Academic Medical Centre, Amsterdam, the Netherlands, and Department of Gastroenterology and Hepatology, Geneva University Hospital, Geneva, Switzerland

J C H Hardwick, Department of Experimental Internal Medicine, and Department of Gastroenterology, Academic Medical Centre, Amsterdam, the Netherlands

Correspondence to: Dr G R van den Brink, Laboratory for Experimental Internal Medicine, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands; g.r.vandenbrink@amc.uva.nl

Conflict of interest: None declared.

REFERENCES

- 1 Potten CS, Booth C, Pritchard DM. The intestinal epithelial stem cell: the mucosal governor. *Int J Exp Pathol* 1997;**78**:219–43.
- 2 Tabata T, Takei Y. Morphogens, their identification and regulation. *Development* 2004;**131**:703–12.
- 3 Korinek V, Barker N, Morin PJ, *et al*. Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. *Science* 1997;**275**:1784–7.
- 4 Nishisho I, Nakamura Y, Miyoshi Y, *et al*. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 1991;**253**:665–9.
- 5 Kinzler KW, Nilbert MC, Su LK, *et al*. Identification of FAP locus genes from chromosome 5q21. *Science* 1991;**253**:661–5.
- 6 Luchtenborg M, Weijenberg MP, Roemen GM, *et al*. APC mutations in sporadic colorectal carcinomas from The Netherlands Cohort Study. *Carcinogenesis* 2004;**25**:1219–26.
- 7 Konishi M, Kikuchi-Yanoshita R, Tanaka K, *et al*. Molecular nature of colon tumors in hereditary nonpolyposis colon cancer, familial polyposis, and sporadic colon cancer. *Gastroenterology* 1996;**111**:307–17.
- 8 Gregorieff A, Clevers H. Wnt signalling in the intestinal epithelium: from endoderm to cancer. *Genes Dev* 2005;**19**:877–90.
- 9 Ireland H, Kemp R, Houghton C, *et al*. Inducible Cre-mediated control of gene expression in the murine gastrointestinal tract: effect of loss of beta-catenin. *Gastroenterology* 2004;**126**:1236–46.
- 10 Korinek V, Barker N, Moerer P, *et al*. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet* 1998;**19**:379–83.
- 11 Pinto D, Gregorieff A, Begthel H, *et al*. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev* 2003;**17**:1709–13.
- 12 Sansom OJ, Reed KR, Hayes AJ, *et al*. Loss of Apc in vivo immediately perturbs Wnt signalling, differentiation, and migration. *Genes Dev* 2004;**18**:1385–90.
- 13 van de Wetering M, Sancho E, Verweij C, *et al*. The beta-catenin/Tcf-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* 2002;**111**:241–50.
- 14 Lees C, Howie S, Sartor RB, *et al*. The hedgehog signalling pathway in the gastrointestinal tract: implications for development, homeostasis, and disease. *Gastroenterology* 2005;**129**:1696–710.
- 15 Nusslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*. *Nature* 1980;**287**:795–801.
- 16 de Santa Barbara P, van den Brink GR, Roberts DJ. Molecular etiology of gut malformations and diseases. *Am J Med Genet* 2002;**115**:221–30.
- 17 Park HL, Bai C, Platt KA, *et al*. Mouse Gli1 mutants are viable but have defects in SHH signalling in combination with a Gli2 mutation. *Development* 2000;**127**:1593–605.
- 18 Bai CB, Joyner AL. Gli1 can rescue the in vivo function of Gli2. *Development* 2001;**128**:5161–72.
- 19 van den Brink GR, Hardwick JC, Nielsen C, *et al*. Sonic hedgehog expression correlates with fundic gland differentiation in the adult gastrointestinal tract. *Gut* 2002;**51**:628–33.
- 20 Nielsen CM, Williams J, van den Brink GR, *et al*. Hh pathway expression in human gut tissues and in inflammatory gut diseases. *Lab Invest* 2004;**84**:1631–42.
- 21 van den Brink GR, Bleumink SA, Hardwick JC, *et al*. Indian Hedgehog is an antagonist of Wnt signalling in colonic epithelial cell differentiation. *Nat Genet* 2004;**36**:277–82.
- 22 Akiyoshi T, Nakamura M, Koga K, *et al*. Gli1, downregulated in colorectal cancers, inhibits proliferation of colon cancer cells involving Wnt signalling activation. *Gut* 2006;**55**:991–9.
- 23 Berman DM, Karhadkar SS, Maitra A, *et al*. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature* 2003;**425**:846–51.
- 24 Oniscu A, James RM, Morris RG, *et al*. Expression of Sonic hedgehog pathway genes is altered in colonic neoplasia. *J Pathol* 2004;**203**:909–17.

HCV

Cellular immune responses against persistent hepatitis C virus: gone but not forgotten

P Klenerman, N Semmo

Failure to sustain antiviral T cell responses is a hallmark of persistent hepatitis C virus infection. Early loss of T cell proliferative capacity after acute disease appears to be an important component of this process. T cell function may, however, be restored in vitro with interleukin 2

Although the prevalence of persistent hepatitis C virus (HCV) infection is very high, acute HCV infection is rarely encountered by most clinicians. The reasons for this include often a relatively mild presentation,

as well as failure to seek medical attention. Nevertheless, analysis of the events which occur during and following acute HCV infection is very important in trying to disentangle why some infections persist and

others are cleared—defining so-called “correlates of protection”. Thus immunological studies of acute HCV are on the one hand potentially extremely informative and on the other very difficult to do.

Acute infection may be followed by rapid clearance or—in the majority of cases—lifelong persistence, often preceded by a period of partial control. Over the past decade a substantial amount of data have been generated pointing to a crucial role for cellular immune responses in determining these outcomes.¹ There is no doubt that a number of factors, including innate responses and neutralising antibody responses, all contribute to host resistance to infection. However, the difficulty in analysing strain specific neutralising antibodies and the great variability in HCV envelope (the target of such antibodies) means that much attention has focused on T cell immunity. The hope is that by reproducing T cell immune responses associated with successful outcomes it might be possible to

generate successful vaccines for protection or immune based therapy.

Previous studies of cellular immune responses during and after acute HCV infection have demonstrated the following general features:

- (A) CD4+ T cell responses—notably proliferative responses—may be observed in acute infection and are maintained in those who clear the virus. However, they are typically lost in those where viral infection becomes persistent.^{2–4}
- (B) CD8+ T cell responses are induced in most cases of acute infection but are not sustained in those where virus is not cleared.^{5–7}
- (C) Host major histocompatibility complex genotype (both class I and class II) plays a crucial role in determining the antiviral cellular immune responses and hence the long term outcome of infection.^{8–9}

The exact status of the CD4+ and CD8+ T cells in persistent infection is not clear, but if present they are found at low frequency in blood, although somewhat enriched in the liver itself.^{10–11} Thus addressing the mechanisms underlying the apparent downregulation of CD4+ and CD8+ T cell responses associated with persistent HCV infection is crucial in understanding HCV persistence.

The study by Folgori and colleagues¹² in this issue of *Gut* provides further important insight into this phenomenon (see page 1012). The authors tracked acute HCV infection longitudinally in a substantial cohort of Italian patients presenting to participating centres. This represents currently the largest cohort of this kind studied in such detail (31 individuals). Using a panel of overlapping peptides representing a prototype genotype 1b sequence, they analysed the breadth and vigour of the T cell response over time. They observed, as has been shown previously, strong and sustained CD4+ and CD8+ T cell responses in those with acute resolving infection.^{2–3,5} In those where immune control was lost, although T cell responses were induced initially, these declined quite rapidly, and became undetectable using ex vivo assays (for example, interferon γ enzyme linked immunospot (IFN- γ ELISpot)) in the majority of patients.^{6–7,13} Impairment of proliferation was also a marked feature of such responses in the persistently infected group; nevertheless, such responses could be rescued in vitro by addition of interleukin 2 (IL-2). The authors propose that loss of T cell responses in the face of continuing viraemia is due to a failure of proliferation in vivo. Collapse

of such responses could contribute to the failure to resolve infection as in those with resolving infection proliferative responses were maintained over time.

How do these new data help us refine a model of successful and unsuccessful cellular immunity against HCV? Currently, the mechanisms underlying the failure to sustain peripheral T cell responses in chronic infection can be considered as four main groups:

- *Escape*. Mutation in T cell epitopes was clearly demonstrated in chimpanzee models¹⁴ although it is only recently in humans that evidence has been really convincing. Mutations leading to reduced recognition by CD8+ T cells have been observed after acute transmission events in a cohort of acutely infected individuals studied prospectively and in single source outbreaks.^{15–17} It is very likely that most of such escape occurs very early, as judged by data in the animal model and in humans. Nevertheless, escape does not occur in all CD8+ T cell epitopes,^{15–18} and all responses undergo substantial decline in chronic disease so alternative mechanisms for T cell downregulation must play some role. The importance of escape in evasion of HCV specific CD4+ T cell responses is not currently known.
- *Exhaustion*. This term is derived from work originally performed in the murine model of lymphocytic choriomeningitis virus (LCMV).¹⁹ In this model, certain viral strains when given in high doses set up high level persistence with long term viraemia. This was associated with decline and loss of CD8+ and CD4+ T cell responses. The early experiments in this field showed deletion of CD8+ T cells but subsequently different investigators have proposed a sliding scale of “partial” exhaustion with progressive loss of IL-2 secretion, proliferation, IFN- γ secretion, and cytotoxicity occurring, prior to full deletion.²⁰ The mechanism is not fully understood but it is an antigen specific effect and probably results from excessive T cell receptor triggering, perhaps in the absence of sufficient costimulation or help (see below). In HCV, the fact that CD8+ T cell responses may be rescued in vitro with antigen and IL-2²¹ suggests that full exhaustion does not occur, but in many respects this model does describe the evolution of CD8+ T cell responses as reductions in diverse aspects of function have been reported,²² including data in the Folgori study.

- *Helplessness*. Failure to provide adequate CD4+ T cell help in murine models also leads to long term failure to control viral infections such as LCMV.²³ This “helpless” state is associated with the development of CD8+ T cell responses which lack full effector and proliferative function. Failure of CD4+ T cell help in HCV was clearly shown in the current study, as elsewhere, and this could readily explain the status of CD8+ T cell responses. A recent study showed that those CD4+ T cell responses which are maintained in persistent HCV infection typically lack the capacity to secrete IL-2, a crucial helper cytokine.²⁴ Furthermore, addition of IL-2 restored functionality to these populations. Thus failure to maintain IL-2 secretion may be a critical factor in the development of a “helpless” state. However, many CD4+ T cell responses do appear to be deleted,^{2–4,24} and in some cases this is an early event, so if failure of CD4+ T cell responses is ultimately responsible for failure of CD8+ T cell responses, this still begs the question of why HCV specific CD4+ T cell responses appear to fail so early.
- *Regulation*. The above models derive largely from work in the murine model of LCMV where escape and exhaustion dominate. In other murine models of virus persistence, such as Friend leukaemia virus, other mechanisms appear important.²⁵ CD25+ and/or Foxp3+ CD4+ regulatory T cells (Tregs) were originally observed in inflammatory conditions but clearly play a role in a variety of infections. Tregs may be “natural” or induced after prolonged antigenic stimulation. It is very likely that they play an important role in preventing immune mediated pathology during chronic HCV. Studies of blood and liver from a variety of groups have demonstrated regulatory activity which can influence the proliferation of CD8+ and CD4+ T cells in vitro.^{26–29} The major issue in understanding the role of Tregs in this disease is that, by and large, the defect in immune responsiveness is HCV specific whereas other peripheral immune responses are left intact. One possible explanation is that although demonstrable in blood, Treg activity is likely to be concentrated at the site of disease (that is, the inflamed liver). Further studies of this issue are required to establish to what extent local Treg activity protects against immunopathology and whether it contributes to the disappearance of HCV specific CD8+ and CD4+ T cells observed in most cohorts.

Overall, the data in the study by Folgori and colleagues¹² add weight to the concepts of exhaustion and helplessness as important factors in HCV specific T cell failure. Sustained exposure of T cells to viral antigen drives them initially into an "effector" state where antiviral cytokines such as IFN- γ and tumour necrosis factor α are secreted but IL-2 secretion is lost. Failure to secrete IL-2 may then contribute to a vicious circle where HCV specific CD8+ and CD4+ responses are induced but proliferation cannot be maintained. Escape of virus from key immune responses by mutation at an early stage will serve to maintain high levels of antigen and accelerate this process. Ultimately, unless virus is cleared rapidly, cells progress along a downward slope to full exhaustion. Importantly, however, although all of these processes may contribute to the decline of antigen specific T cell responses, it is still rather difficult to disentangle what is a cause and what is a consequence of virus persistence.

One further element must be considered, which is difficult to address in studies of acute HCV but which is nevertheless probably critical. The liver has an important role as an immune regulator, containing specialised antigen presenting cells and populations of non-classical T cells and natural killer cells with potential antiviral and regulatory functions. Virus is also able to replicate within the liver for weeks or months before the initiation of adaptive immune responses. To what extent liver specific factors play a role in the downregulation of cellular immune responses in acute HCV infection has yet to be determined. However, the fact that despite such a tolerising environment (in addition to all of the mechanisms outlined above) a substantial proportion of individuals do go on to clear virus provides hope that tweaking the system in favour of the T cell may provide an escape route leading to resolution of viral infection. Whether IL-2, as suggested by the data of Folgori and others, may be the key to open this escape route is an intriguing idea, and perhaps one which could be considered in vivo in future.

ACKNOWLEDGEMENTS

PK and NS receive funding from the Wellcome Trust, the Fritz Thyssen Foundation, the European Union (VIRGIL network), the Medical Research Council, and the James

Martin School, University of Oxford. Thanks go to Ellie Barnes, Gillian Harcourt, and Scott Ward for continued input into studies of and discussions about cellular immunity against HCV.

Gut 2006;55:914–916.

doi: 10.1136/gut.2005.087304

Conflict of interest: declared (the declaration can be viewed on the Gut website at <http://www.gutjnl.com/supplemental>).

Authors' affiliations

P Klenerman, N Semmo, Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, Oxford, UK

Correspondence to: Dr P Klenerman, Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, Oxford OX3 9TU, UK; paul.klenerman@ndm.ox.ac.uk

REFERENCES

- Bowen DG, Walker CM. Adaptive immune responses in acute and chronic hepatitis C virus infection. *Nature* 2005;436:946–52.
- Diepolder HM, Zachoval R, Hoffmann RM, et al. Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. *Lancet* 1995;346:1006–7.
- Missale G, Bertoni R, Lamonaca V, et al. Different clinical behaviours of acute HCV infection are associated with different vigor of the anti-viral T cell response. *J Clin Invest* 1996;98:706–14.
- Day CL, Seth NP, Lucas M, et al. Ex vivo analysis of human memory CD4 T cells specific for hepatitis C virus using MHC class II tetramers. *J Clin Invest* 2003;112:831–42.
- Lechner F, Wong DK, Dunbar PR, et al. Analysis of successful immune responses in persons infected with hepatitis C virus. *J Exp Med* 2000;191:1499–512.
- Lechner F, Gruener NH, Urbani S, et al. CD8+ T lymphocyte responses are induced during acute hepatitis C virus infection but are not sustained. *Eur J Immunol* 2000;30:2479–87.
- Cox AL, Mosbruger T, Lauer GM, et al. Comprehensive analyses of CD8+ T cell responses during longitudinal study of acute human hepatitis C. *Hepatology* 2005;42:104–12.
- Thursz M, Yallop R, Goldin R, et al. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C European Network for Cooperative Research. *Lancet* 1999;354:2119–24.
- McKiernan SM, Hagan R, Curry M, et al. Distinct MHC class I and II alleles are associated with hepatitis C viral clearance, originating from a single source. *Hepatology* 2004;40:108–14.
- He X-S, Rehmann B, Lopez-Labrador F, et al. Quantitative analysis of HCV-specific CD8+ T cells in peripheral blood and liver using peptide-MHC tetramers. *Proc Natl Acad Sci USA* 1999;96:5692–7.
- Grabowska AM, Lechner F, Klenerman P, et al. Direct ex vivo comparison of the breadth and specificity of the T cells in the liver and peripheral blood of patients with chronic HCV infection. *Eur J Immunol* 2001;31:2388–94.
- Folgori A, Spada E, Pezzanera M, et al. Early impairment of hepatitis C virus specific T cell proliferation during acute infection leads to failure of viral clearance. *Gut* 2006;55:1012–9.
- Lauer G, Barnes E, Lucas M, et al. High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection. *Gastroenterology* 2004;127:924–36.
- Erickson AL, Kimura Y, Igarashi S, et al. The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. *Immunity* 2001;15:883–95.
- Cox AL, Mosbruger T, Mao Q, et al. Cellular immune selection with hepatitis C virus persistence in humans. *J Exp Med* 2005;201:1741–52.
- Timm J, Lauer GM, Kavanagh DG, et al. CD8 epitope escape and reversion in acute HCV infection. *J Exp Med* 2004;200:1593–604.
- Ray SC, Fanning L, Wang XH, et al. Divergent and convergent evolution after a common-source outbreak of hepatitis C virus. *J Exp Med* 2005;201:1753–9.
- Urbani S, Amadei B, Cariani E, et al. The impairment of CD8 responses limits the selection of escape mutations in acute hepatitis C virus infection. *J Immunol* 2005;175:7519–29.
- Moskophidis D, Laine E, Zinkernagel RM. Peripheral clonal deletion of antiviral memory CD8+ T cells. *Eur J Immunol* 1993;23:3306–11.
- Wherry EJ, Ahmed R. Memory CD8 T-cell differentiation during viral infection. *J Virol* 2004;78:5535–45.
- Lauer GM, Ouchi K, Chung RT, et al. Comprehensive analysis of CD8(+) T-cell responses against hepatitis C virus reveals multiple unpredicted specificities. *J Virol* 2002;76:6104–13.
- Wedemeyer H, He XS, Nascimbeni M, et al. Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. *J Immunol* 2002;169:3447–58.
- Zajac AJ, Vance RE, Held W, et al. Impaired antiviral T cell responses due to expression of the Ly49A inhibitory receptor. *J Immunol* 1999;163:5526–34.
- Semmo N, Day CL, Ward SM, et al. Preferential loss of IL-2-secreting CD4+ T helper cells in chronic HCV infection. *Hepatology* 2005;41:1019–28.
- Dittmer U, He H, Messer RJ, et al. Functional impairment of CD8(+) T cells by regulatory T cells during persistent retroviral infection. *Immunity* 2004;20:293–303.
- Sugimoto K, Ikeda F, Stadanlick J, et al. Suppression of HCV-specific T cells without differential hierarchy demonstrated ex vivo in persistent HCV infection. *Hepatology* 2003;38:1437–48.
- Cabrera R, Tu Z, Xu Y, et al. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. *Hepatology* 2004;40:1062–71.
- Boettler T, Spangenberg HC, Neumann-Haefelin C, et al. T cells with a CD4+CD25+ regulatory phenotype suppress in vitro proliferation of virus-specific CD8+ T cells during chronic hepatitis C virus infection. *J Virol* 2005;79:7860–7.
- Rushbrook SM, Ward SM, Unitt E, et al. Regulatory T cells suppress in vitro proliferation of virus-specific CD8+ T cells during persistent hepatitis C virus infection. *J Virol* 2005;79:7852–9.