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Role of hepatitis B virus specific cytotoxic T cells in liver damage and viral control

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Abstract

To understand the role of cytotoxic T cells in liver damage and viral control, we used human histocompatibility leukocyte antigen (HLA)-peptide tetramers that allow direct ex vivo quantification of circulating and liver-infiltrating HBV-specific CD8 cells. Studies were carried out in two groups of patients, one without liver inflammation and minimal HBV replication and the other with liver damage and inflammation along with a high level of viral replication. Contrary to expectation, a high frequency of intrahepatic HBV-specific CD8 cells was found in the former group, i.e., the absence of hepatic immunopathology. In the replicating viraemic group, the virus specific T cells were diluted among the liver infiltrates; although with the massive cellular infiltration that was present, the absolute number was similar. It was also shown that in the low viraemia group the reservoir of CD8+ cells present in the circulation was able to expand after specific virus recognition and that this was not detectable in highly viraemic patients with liver inflammation.

These results show that inhibition of virus replication can be independent of liver damage and when the HBV-specific CD8 response is unable to control virus replication it may contribute to liver pathology not only directly but by causing recruitment of non-virus specific T cells.

Keywords: HBV; CD8 cells

Hepatitis B is not a directly cytopathic virus and the ability to mount an efficient helper and cytotoxic T cell response against it, is thought to be critical in determining the outcome of the infection in terms of both liver damage and virus control. The mechanism by which cytotoxic T lymphocytes (CTLs) cause liver damage is thought to be direct lysis of hepatocytes. In the chronic low viraemia state often referred to as a healthy carrier in which liver damage is minimal, the CTLs are thought to have become anergic or to have been clonally deleted. However, in the past few years several groups have shown that when viruses infect hepatocytes they are more likely to be controlled by intracellular activity mediated by cytokines rather than by direct killing of infected cells. Thus, Chisari and co-workers have clearly shown that HBV replication is completely abolished in the hepatocytes of HBV transgenic mice by cytokine dependant pathways that do not require cell death (Guidotti and Chisari, 1986).

To date, studies have been hampered by the difficulty of determining the intrahepatic compartment of CTLs. Knowledge of the HBV-specific CTL response has been mostly restricted to the circulatory component. The low level of virus specific CD8 positive cells in the circulation of chronically infected HBV patients has been partially reconciled with the model of liver damage mediated by HBV-specific cytotoxic CD8 cells, on the assumption that HBV-specific CTLs are preferentially sequestered in the liver where they cause persistent hepatic damage.

1. Material and methods

As shown in Fig. 1, in the present study on the relationship between the immune mediated reaction and the occurrence of liver damage and viral replication, quantitative measurements of HBV-specific CD8 cells have been determined both in the circulation and intrahepatic compartment (Maini et al., 2000). This has been made possible by the development of the technique of HLA peptide tetramer complexes that allow direct visualization of CD8 positive cells specific for HBV antigens. The multimeric HLA class-I peptide complex has a high affinity for T cells displaying the appropriate T cell receptor. Binding to the specific cells is quantified by cytofluorimetric analysis using a

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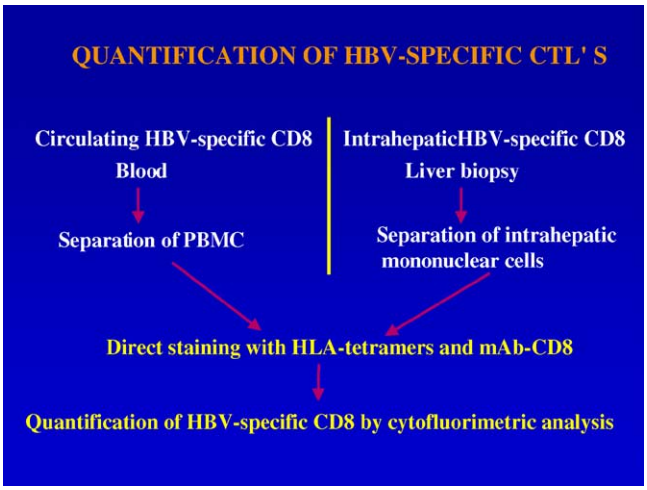

Fig. 1. Methodology for quantification of HBV-specific CTLs in different compartments.

fluorochrome—labelled streptavidin reagent. The frequency of both HBV-specific CD8 positive cells was determined by the tetramer technique after separation of peripheral blood mononuclear cells from blood samples and in liver biopsies after separation of intrahepatic mononuclear cells.

Two groups of HBV positive patients differing in the extent of liver damage and viral control was investigated. One group included patients in the actively replicating phase of the disease with HBe Ag positivity, high HBV DNA level (>800 μg/ml) and with evidence of liver cell damage as shown by an elevated serum AST. The other group had minimal evidence of liver inflammation, namely absent HBe Ag and low HBV DNA level (<2 μg/ml).

2. Results

As shown in Fig. 2, the frequency of HBV-specific CD8 cells in the circulation was determined by the tetramer technique using three different HBV synthetic peptides corresponding to the sequence of the epitopes core 18–27, envelope 335–343 and polymerase 575–583 region of HBV. The number of tetramer binding cells is clearly different in the two groups of chronically infected HBV patients. In most of the 10 patients without serum ALT elevations and low viral replication, the frequency of tetramer cells exceeded the 0.02% of circulating CD8 positive cells which represent the maximum detection observed in the two control groups. Nine of these ten patients had specificity demonstrated for more than one epitope. In contrast, the frequency of tetramer positive cells in HBV patients with high ALT and HBV DNA levels showed a frequency above that of the controls in two patients only.

The lower percentage of HBV-specific CD8 positive cells in the circulation of patients in the latter group could be the result of preferential compartmentalization within the liver. Fig. 3 illustrates the results for core peptide specific CD8 cells in both liver and blood for two patients, one from each group. Although the HBV-specific CD8 cells are preferentially sequestered in the liver, in the patient example with high serum ALT and HBV DNA levels, the frequency of such cells as a percentage of the total intrahepatic T cells purified from the biopsy specimen was not high. In fact at 0.23%, it was considerably lower than the 4% frequency of CD8 positive cells found in the other patient example shown who had a low serum transaminase and HBV DNA levels. In other words, the higher intrahepatic frequency was found in the absence of biochemical evidence of hepatocyte damage.

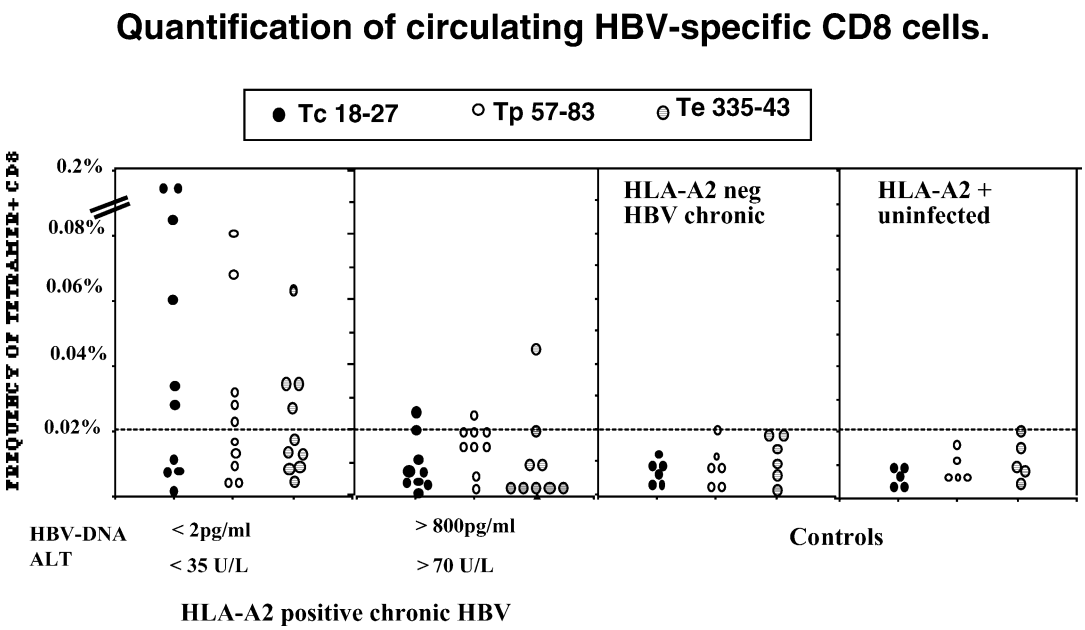

Fig. 2. Results of quantification for HBV-specific CD8 cells in circulation (from data of Maini et al., 2000), including results from two control groups.

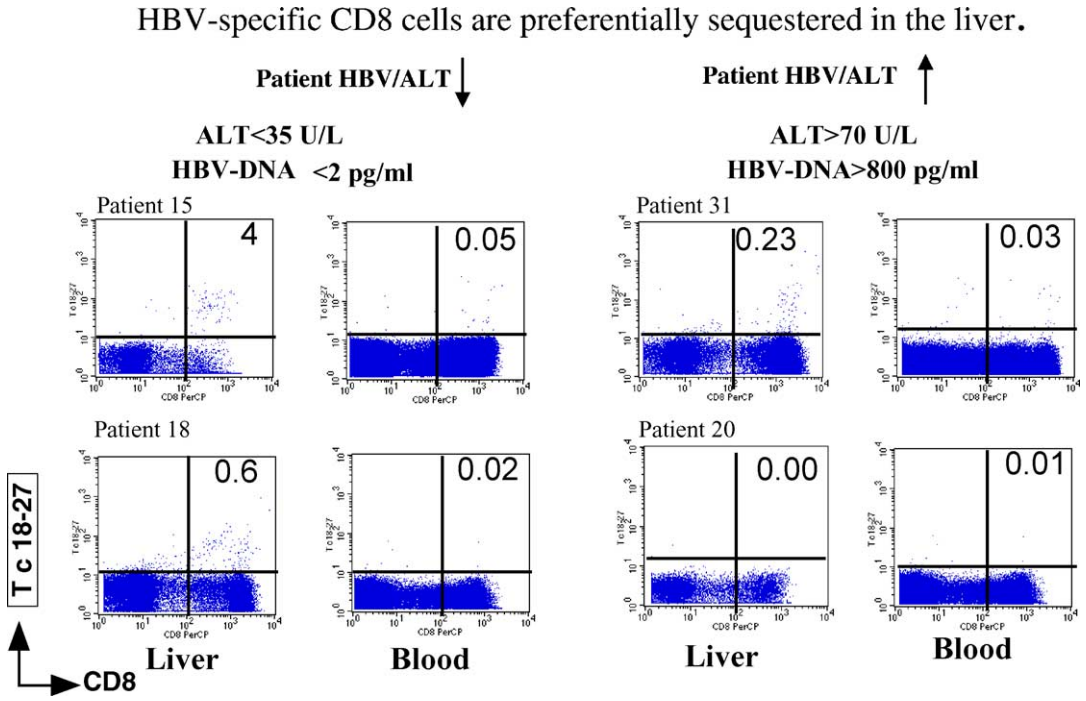

Fig. 3. FACS dot plots of liver and blood isolates from two patients, one with and one without liver damage (from data of Maini et al., 2000).

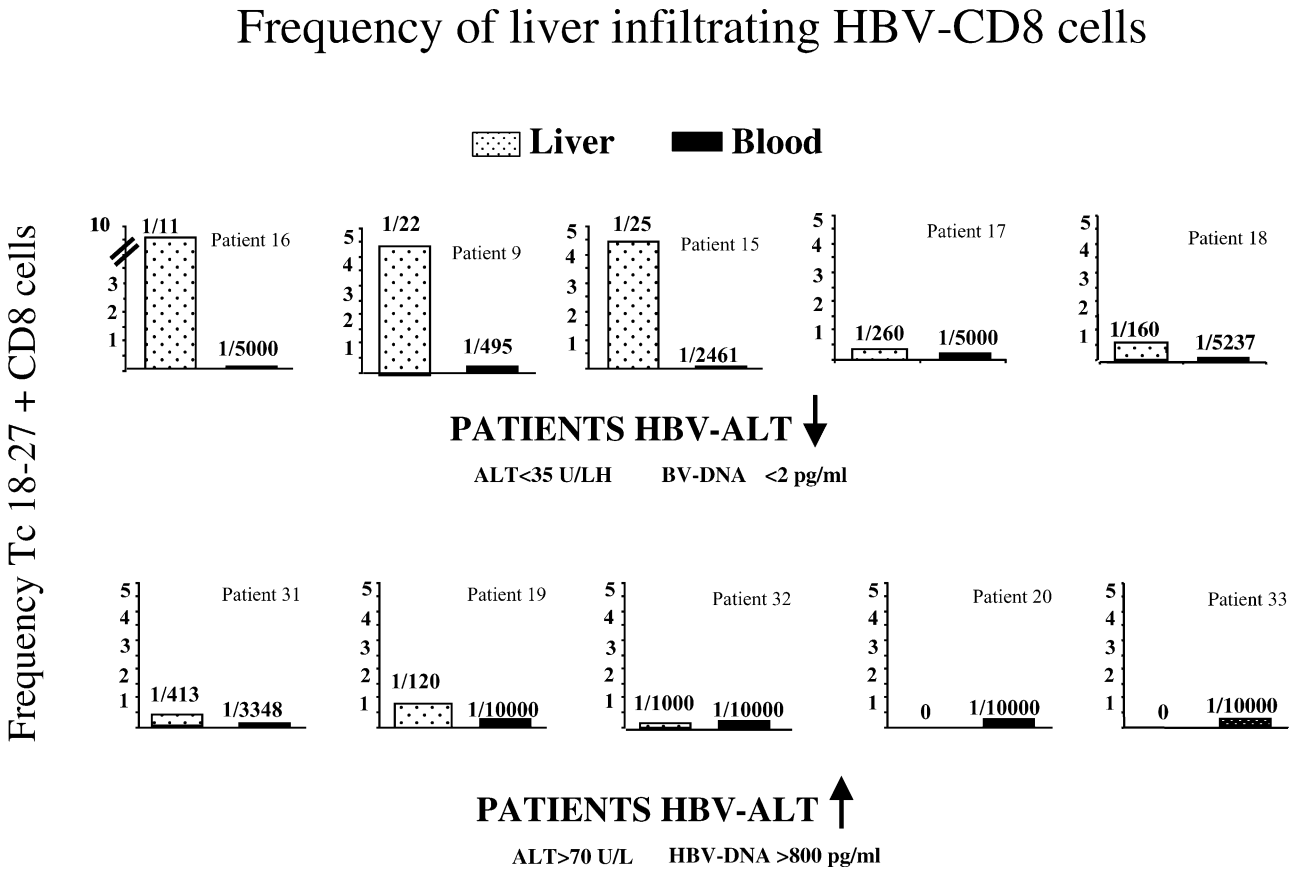

Fig. 4. Frequency of liver and circulating $TC_{18–27}$ CD8 cells assessed by flow cytometric analysis. Bars represent percentages of total CD8 cells and numbers on the top of the bars, the ratio of $TC_{18–27}$ CD8+ cells to total CD8+ cells (from data of Maini et al., 2000).

A comparison of the results for the frequency of HBV CD8 cells specific for core epitope in five patients from each of the two groups is given in Fig. 4. The lower intrahepatic percentage in those with evidence of liver damage is shown.

The different frequency of core epitope positive CD8 cells could reflect differing degrees of dilution in tetramer negative CD8 cells, rather than a difference in the absolute number of HBV-specific CD8 positive cells. To better quantify the number of liver-infiltrating tetramer positive cells, an immunohistological comparison of the total CD8 infiltration to the liver sections was also performed. These numbers were then correlated with the frequency of core peptide positive CD8 cells obtained in the purified infiltrate. The immunostaining was carried out in formalin fixed

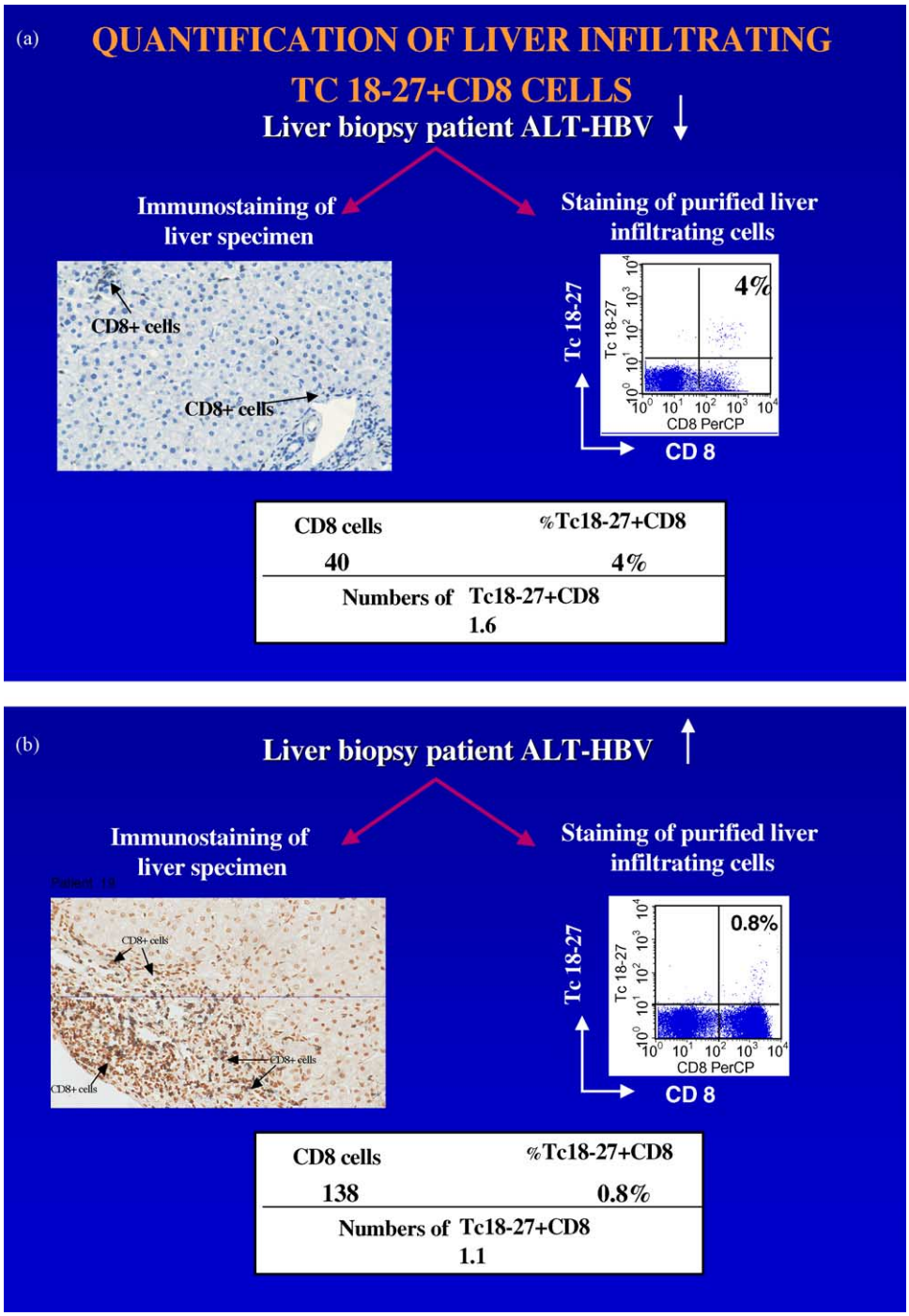

Fig. 5. Analysis of liver-infiltrating CD8 cells by immunostaining giving number of CD8+ CTLs in portal tracks and intralobular areas. By multiplying figures of $TC_{18–27}$ + positive cells purified from same biopsy, total number in each field obtained, (a) minimal liver damage and (b) liver damage and high viral load (from data of Maini et al., 2000).

paraffin embedded biopsies and the number of CD8 positive T lymphocytes in portal tracks and intralobular areas scored in equivalent histological fields. The number was then multiplied by the frequency of core peptide positive cells obtained from CD8 positive cells purified freshly from the same biopsy to estimate the total number of peptide positive cells in each high power field, as shown in Fig. 5a and b. As expected the subjects with normal hepatic enzymes had relatively low numbers of infiltrating CD8 cells preferentially localized within the lobules. In contrast, for patients with elevated liver enzymes, a large number of CTLs were present in the portal areas with some spreading into the intralobular areas. Although frequency of core epitope positive CD8 cells is low, as previously shown, the total number of these—because of the larger size of the infiltrate—was not significantly different from the patients without liver damage. The quantification was similar for other patients examined from the two groups by both the techniques of immunostaining and tetramer positive frequency.

These data, therefore, show that similar numbers of intrahepatic core epitope CD8 positive cells can be associated with different clinical outcomes. Patients with good control of their HBV infection have a relative paucity of CD8 infiltrates composed of a high percentage of core epitope positive cells. By contrast patients with viral control and liver damage are characterised by a much greater infiltrate of CD8 positive cells in which the core epitope positive fraction appear more diluted.

3. Discussion

The findings of a large non-virus specific component of the CD8 liver infiltrate in those with active liver damage are consistent with studies in a transgenic mouse model of fulminant hepatitis showing recruitment of non antigen specific CD8 cells mediated by interferon gamma (Ando et al., 1993). Similarly, in chimpanzees infected with HBV, liver damage occurs concomitantly with a massive infiltration of non antigen specific CD8 cells (Guidotti et al., 1999).

With respect to the control of viral replication, these findings are in accord with the results of studies on CTLs in other viral infections. Thus, HCV specific CTLs can be demonstrated in the normal liver of chimpanzees one year after resolution of an acute HCV infection. In the mouse model of influenza virus infection, efficient virus control is dependent on the kinetics and distribution of the virus specific CD8 response and is not associated with pathological damage induced by the immune response. The pattern of the HBV-specific CD8 response is also similar to that seen in other human persistent virus infections such as EBV, and HTLV-1 in which a robust CTL response can play an important role in limiting virus replication without causing inflammatory disease (for references, see paper of Maini et al. (2000) and review of Bertoletti and Maini (2000)). Our data do not establish whether HBV-specific CD8 cells can control virus replication through the secretion of cytokines alone or whether direct lysis of infective cells is also involved. The sparsely scattered pattern of CD8 positive cells within the liver parenchyma of patients with low viral replication suggests that secreted cytokines may be playing a major role in antiviral control. Recent demonstration of the efficacy of IFN-γ in activating a pathway of intracellular virus inactivation in the hepatocytes is further evidence of this interpretation. However, some degree of direct hepatocyte lysis caused by the HBV-specific CD8 cells may exist which is not detectable by serum liver enzyme measurement.

Further results not included in this paper have shown that in the patients capable of controlling the virus, the phenotype of the circulating HBV-specific CD8 cells was that of Ag-experienced resting cells. These cells exhibit efficient proliferation after re-encounter with the viral Ag and can exert antiviral effector functions after such expansion. This reservoir of circulating HBV-specific CD8 cells able to expand after recognition of the specific virus sequence is not detectable in patients unable to control the virus (Maini et al., 2000).

The presence of similar numbers of virus specific CD8 cells, despite large differences in viral load, seems counterintuitive, if CTLs have an important role in viral control. However, this apparent conundrum has also been observed in HTLV-1 and fits the mathematical model where steady state CTL numbers do not correlate with virus load (Nowak and Bangham, 1996). The model suggests instead that the primary factor controlling viral load is CDL responsiveness, which denotes the rate at which virus specific CD8 cells expand and exert anti-viral activity. Since sufficient CTL responsiveness will result in the lowering of the viral load, viral specific CD8 cells would fall with the lower antigen stimulus. Thus, at equilibrium there may be little discernable difference in actual CTL abundance between patients with high and low levels of HBV replication. The findings in our two groups of HBV infected patients are consistent with this model in that the most striking difference between them is their CTL responsiveness rather than actual numbers at equilibrium.

4. Conclusion

(1) In patients lacking evidence of liver damage and controlling viral replication, core specific CD8s are present in circulation and within the liver, demonstrating that an active cellular response to HBV is not lacking in these subjects and that liver damage and viral control are independently determined.
(2) In patients with liver inflammation and high level of HBV replication, there is a different pattern of HBV-specific CD8s which are low or undetectable in circulation and diluted in the liver by a much greater infiltration of non-specific T cells which are probably responsible for the hepatocyte lysis.

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