Predominant T-Helper 1 Cytokine Profile of Hepatitis B Virus Nucleocapsid-Specific T Cells in Acute Self-Limited Hepatitis B

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The cytokine pattern secreted by T cells on viral antigen recognition is believed to exert a profound influence on both the type of disease caused by the infecting agent and the final outcome of the viral infection. To characterize the cytokine pattern associated with spontaneous resolution of acute hepatitis B, we analyzed interferon gamma (IFN-γ), interleukin (IL)-4, and IL-5 production by a wide series of hepatitis B virus (HBV) nucleocapsid-specific T-cell lines (34 lines) and T-cell clones (71 clones) derived from the peripheral blood of 13 patients during the acute or recovery phase of hepatitis B (2 and 7 of them were studied only in the recovery or the acute phase, respectively, and 4 during both). Most T-cell lines (67%) and clones (77%) isolated during the acute phase of infection expressed a T-helper (Th) 1 cytokine profile dominated by the production of IFN-γ. A larger proportion (74%) of T-cell lines produced several years after resolution of hepatitis was able to secrete not only IFN-γ, but also IL-4 and IL-5 (Th0-like cells). Results indicate that the antigen-specific fraction of peripheral blood T cells in acute self-limited hepatitis B selectively secrete Th1-type cytokines, suggesting that Th1-mediated effects may contribute not only to liver cell injury, but probably also to recovery from disease and successful control of infection. (Hepatology 1997;25:1022-1027.)

Patients with acute self-limited hepatitis B are able to mount vigorous HLA class I[1-3] and class II[4,5] restricted T-cell responses against different epitopes within hepatitis B virus (HBV) antigens, whereas patients with chronic HBV infection and persistent viral replication show weaker and frequently undetectable HBV-specific T-cell responses.[6,7] This different vigor of the antiviral T-cell response is believed to represent a crucial determinant of the final outcome of infection. However, the mechanisms responsible for this difference are still largely undefined.[8]

Besides quantitative differences, qualitative changes of the immune response may also play a crucial role in determining resolution or persistence of infection. In particular, the cytokine profile stimulated by the initial encounter with the virus can either contribute to efficiently inhibit viral replication if Th1 helper (Th) cytokines are predominant, or facilitate the spread of the pathogen within the host if the balance is in favor of T-2-type cytokines.[9,10] Indeed, it has been shown that Th1 cytokines (such as interleukin [IL]-2, interferon gamma [IFN-γ], and tumor necrosis factor β) are principally involved in cell-mediated immunity[11]; they can contribute to the immunopathology caused by the infecting agent, and can also play a crucial role in protection from intracellular pathogens,[12,13] including viruses.[14,15] In contrast, Th2 cytokines (including IL-4 and IL-10) can be beneficial against extracellular pathogens,[16] because they mostly regulate humoral immune responses, but their predominant effect can be associated with progressive disease by intracellular infectious agents.[11-13]

To characterize the cytokine profile associated with acute self-limited hepatitis B and successful resolution of disease, HBV nucleocapsid-specific T-cell lines and clones were produced from the peripheral blood of patients acutely infected with HBV or recovered from acute hepatitis, and were tested for their cytokine secretion on antigen or mitogen stimulation. Results indicate that most circulating HBV nucleocapsid-specific T lymphocytes in acute HBV infection are Th1-like cells that may contribute not only to liver injury, but also to create conditions favorable to the eradication of viral infection.

PATIENTS AND METHODS

Patients. Eleven patients (2 females and 9 males; mean age, 37.3 $\pm$ 12.2 years) with acute self-limited hepatitis type B were studied. The diagnosis of acute hepatitis was based on the finding of elevated values of serum glutamic pyruvic transaminase activity (at least 10 times the upper level of normal), associated with the detection of immunoglobulin (Ig)M anti hepatitis B core antigen antibodies in the serum, as well as the recent onset of jaundice and other typical symptoms of acute hepatitis. All patients were negative for antibodies to hepatitis C virus and to HIV-1,2 with the exception of a patient who was positive for hepatitis C virus antibodies. Four of them were also studied 5 to 6 years following clinical recovery. Two additional patients were studied only after resolution (>6 years) of hepatitis (i.e., they were negative for hepatitis B surface and e antigens, and positive for antibodies to hepatitis B core (HBcAg) and surface antigens with persistently normal aminotransferase levels).

Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the human research review commitee of the University of Parma.

HBV Antigens and Synthetic Peptides. A recombinant preparation of HBcAg was obtained from bacterial extracts of *Escherichia Coli* K12 strain HB 101 harboring the recombinant plasmid carrying the HBcAg coding gene as described previously.[17] Purity was over 95% as determined by scanning densitometry of Coomassie Blue stained sodium dodecyl sulfate–polyacrylamide gel.

An HBcAg deletion mutant lacking the carboxyl-terminal 39 amino acid residues of the core molecule was produced in *E. Coli*

Abbreviations: HBV, hepatitis B virus; Th, T helper; IL, interleukin; IFN-γ, interferon gamma; Ig, immunoglobulin; HBcAg, hepatitis B core antigen; PBMC, peripheral blood mononuclear cells; APC, antigen-presenting cells; PHA, phytohemagglutinin.

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and provided by Biogen (Geneva, Switzerland).[18] Its purity was 99.8% and it is herein designated as hepatitis B e antigen.

Twenty-two partially overlapping peptides, 10 to 20 amino acids long, corresponding to the complete sequence of the core region–encoded polypeptide (subtype ayw), were purchased from Multiple Peptide System (La Jolla, CA) and Chiron Mimotopes (Clayton, Victoria, Australia), or kindly provided by Cytel (San Diego, CA). Amino acid sequences of the synthetic peptides used in this study, and indicated by the amino acid position from the N-terminus of the core derived polypeptide, are as follows: 1-20, 11-27, 20-34, 28-47, 38-54, 50-59, 50-64, 50-69, 61-80, 70-89, 82-101, 91-110, 100-119, 111-125, 117-131, 120-139, 131-145, 140-155, 147-161, 155-169, 162-176, and 169-183.

Isolation of Antigen-Specific Polyclonal T-Cell Lines and T-Cell Clones. Peripheral blood mononuclear cells (PBMC) isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation and resuspended in RPMI 1640 supplemented with 25 mmol/L HEPES, 2 mmol/L L-glutamine, 50 μg/mL gentamycin, and 8% human serum (complete medium) were cultured either in round-bottom wells of 96-well plates (Costar, Cambridge, MA) at 1×10^6 cells per milliliter or in 24-well plates (Costar) at 2.5 to 3×10^6 cells per 1.5 mL in the presence of hepatitis B e antigen or HBcAg (0.5 μg/mL) or individual synthetic peptides (1 μg/mL). After 5 to 7 days at 37°C in an atmosphere of 5% CO_2 in air, activated T cells were expanded by adding recombinant IL-2 (20 U/mL; Cetus Corporation, Emeryville, CA). On day 14, growing lines were tested for antigen specificity and those that scored positive were restimulated with antigen or peptide in the presence of irradiated (4,000 rads) autologous PBMC (5×10^5/mL) as a source of antigen-presenting cells (APC) in medium supplemented with IL-2. From this point, T-cell lines were kept in culture by restimulation with antigen or peptide in the presence of autologous-irradiated APC every 10 to 14 days and provided with supplementary IL-2–containing medium between restimulations to maintain the cell concentration between 3×10^5 and 1×10^6.[4]

Selected antigen-specific T-cell lines were cloned by limiting dilution at 1 cell per well in round-bottom 96-well plates, in RPMI medium containing 10% fetal calf serum, 3% human serum, and IL-2 (20 U/mL), in the presence of allogeneic-irradiated (6,000 rads) PBMC as feeder cells (1×10^5 cells per well) and phytohemagglutinin (PHA) (1:200; PHA-M, Gibco, Life Technologies, Paisley, UK). All wells were restimulated weekly with irradiated feeder cells and IL-2 until growing colonies were tested for antigen specificity. Specific T-cell clones were restimulated every 20 days by adding irradiated allogeneic feeder cells (5×10^5/mL) and PHA (1:400), and were provided with supplementary IL-2–containing medium between restimulations.

The probability for each positive well being a clone derived from a single precursor was calculated by means of a conditional probability argument assuming Poisson statistics.[19]

Proliferation Assay. For the study of polyclonal T-cell lines and clones, T cells were washed extensively to remove IL-2 and fetal calf serum, and subsequently cocultured in 96-well plates (2 to 3×10^4/well), in complete medium, for 3 days with autologous irradiated PBMC (1×10^5/well) as APC in the presence or absence of antigens or peptides.

All proliferation assays were performed in triplicate in round-bottom 96-well plates, and ^{3}H-thymidine (0.5 μCi per well; specific activity, 2.0 Ci/mmol; Amersham International, Amersham, Little Chalfont, Buckinghamshire, UK) was added 6 hours before harvesting. The results are expressed as a stimulation index, which represents the ratio between mean counts per minute obtained in the presence of antigens or peptides and the counts per minute measured in the absence of antigens or peptides. Stimulation index values above 4 were required to define a proliferative response as positive.

HLA Restriction of Antigen Recognition. The HLA restriction of peptide recognition by T cells was determined using either murine monoclonal antibodies against human HLA class I and class II molecules to block cell proliferation or allogeneic APC (HLA matched or mismatched with the T-cell donor) to present the peptide to T-cell lines or clones.

Monoclonal antibodies recognizing monomorphic determinants of HLA DR (D1-12; IgG2a), DQ1 (BT3/4; IgG1), DQ2 (XIII358/4; IgG2a), DQ3 (XIV466; IgG2b), DP (B7/21; IgG2a), and a monoclonal antibody specific for HLA-A, -B, and -C antigens (W6/32; IgG2a) was a gift from Dr. R. S. Accolla, University of Genova, Italy.[4] Anti–HLA class II monoclonal antibodies were used at a final dilution of ascitic fluid of 1:500; purified W6/32 was used at the concentration of 1 μg/mL. Autologous irradiated APC were incubated first with monoclonal antibodies for 1 hour at 37°C, then with synthetic peptides (0.5 μg/mL, 1 hour, 37°C) or with HBV antigens (0.5 μg/mL, 4 to 6 hours, 37°C), and finally washed before addition to responder T cells. Results are expressed as a percentage of inhibition of the proliferation (measured as ^{3}H-thymidine incorporation) obtained in the absence of anti-HLA monoclonal antibodies.

Allogeneic irradiated antigen presenting cells were incubated for 2 hours at 37°C in medium alone or with different concentrations of synthetic peptides, washed extensively and then cocultured (1×10^5 cells per well) for 3 days with responder T cells. ^{3}H-Thymidine was added to the cultures 6 hours before harvesting.

Cytokines Production and Quantitation. T cells from antigen-specific lines (1×10^6/mL) were washed extensively and incubated with an equal number of autologous irradiated APC in the presence of antigen or peptides. T-cell blasts of each clone (1×10^6/mL) were stimulated either with PHA alone (1:200) or with antigen or peptide in the presence of autologous irradiated APC. After 40 to 48 hours of culture, T-cell blasts were centrifuged and the supernatants were collected and stored at −70°C. IFN-γ and IL-4 were quantitated by commercially available enzyme-linked immunosorbent assays (BioSource International Inc., Camarillo, CA, and Quantikine R & D Systems, Minneapolis, MN, respectively), as previously described.[20] For the measurement of IL-5, the murine LyH7.B13 cell line was used as a source of indicator cells.[20] A semiquantitative estimate of IL-5 produced by T cells was obtained by a standard curve of human recombinant IL-5 (Genzyme, Cambridge, MA). Values of the cytokine content 5 SD over those of control supernatants derived from irradiated feeder cells alone were regarded as positive.

TABLE 1. Fine Specificity of the Polyclonal T-Cell Lines and Clones Produced by PBMC Stimulation With HBV Nucleocapsid Antigen or Peptides From 11 Patients With Acute Hepatitis B

Peptide (AA Sequence)	Number of T-Cell Populations Responsive to the Indicated Peptides		Responsive Patients
	T-Cell Lines	T-Cell Clones	
1-20	5	1	5
20-34	1	14	1
28-47	4	5	4
50-69	6	10	6
91-110	4	0	4
100-119	2	28	2
111-125	3	0	3
117-131	4	3	4
120-139	2	0	2

NOTE. The fine specificity of 3 HBcAg-specific lines and 10 HBcAg-specific clones could not be defined with the available panel of peptides. While each T-cell clone was able to recognize only one peptide, polyclonal lines produced by PBMC stimulation with protein were all multispecific, i.e., able to recognize more than one peptide.

Abbreviation: AA, amino acid.

RESULTS

HBV Nucleocapsid-Specific T-Cell Lines and Clones. PBMC isolated during the acute phase of infection from 11 patients with self-limited hepatitis B were stimulated either with HBV nucleocapsid antigen or with HBV core synthetic peptides to expand HBV-responsive T cells. Fifteen antigen-specific polyclonal T-cell lines were derived by HBcAg (9 lines) or peptide stimulation (6 lines).

The fine specificity of 6 of the 9 T-cell lines produced by PBMC stimulation with antigen was then identified using synthetic peptides (Table 1). All were able to recognize more than one peptide. On the other hand, all 6 T-cell lines produced with synthetic peptides also responded to the whole recombinant core antigen, showing that the immunogenic epitopes could actually be generated by the intracellular processing of HBcAg (data not shown).

To compare the functional features of HBV-specific T cells circulating in the acute and recovery phases of hepatitis, 19

TABLE 2. HLA Restriction of Antigen Recognition by HBV Nucleocapsid-Specific T Cells

Fine Specificity	Responder T Cells	Restriction Method	HLA Restriction	% Inhibition DR	% Inhibition DQ	% Inhibition DP
1-20	CF line	MoAb	DR	75	0	0
11-27	LA line	MoAb	DQ	0	85	25
18-27	RA line 1	MoAb	DP	0	0	91
28-47	RI line	Allo APC	DR07			
	VD line	Allo APC	DQ0502			
50-64	SM line 1	MoAb	DP	0	0	85
50-69	SM line 2	MoAb	DP	8	0	90
	CA line	Allo APC	DQ0503			
	GP line	Allo APC	DR0301			
91-110	RA line 1	MoAb	DQ	0	62	0
111-125	SM line 3	MoAb	DP	15	15	75
	RA line 1	MoAb	DQ	0	45	0

NOTE. T-cell lines of the indicated fine specificity were stimulated with immunogenic peptides either in the presence of HLA-matched or -mismatched APC or in the presence of autologous APC with or without anti-HLA monoclonal antibodies, as described in Patients and Methods. No inhibition was observed with anti–HLA class I monoclonal antibodies.

Abbreviations: MoAb, monoclonal antibody; Allo, allogenic.

polyclonal T-cell lines were produced by antigen or peptide stimulation of PBMC isolated several years after the complete clinical resolution of disease. Five of the 19 lines were produced with antigen, and, in 4 of them, the fine specificity was defined by stimulation with synthetic peptides.

Seven polyclonal T-cell lines derived from four patients during the acute phase of infection (4 of known peptide specificity and 3 able to recognize recombinant core antigen) were selected for cloning by limiting dilution. A total of 71 HBV nucleocapsid-specific $CD4^+$ T-cell clones were obtained: 10 proliferated to HBV nucleocapsid antigens, but no peptide specificity could be defined; 1 was specific for HBc_{1-20}, 14 for HBc_{20-34}, 5 for HBc_{28-47}, 10 for HBc_{50-69}, 28 for $HBc_{100-119}$, and 3 for $HBc_{117-131}$ (Table 1).

Blocking experiments with anti-HLA monoclonal antibodies and presentation of the immunogenic peptides by HLA-matched or -mismatched APC showed that most HBV nucleocapsid epitopes identified in our study were recognized by T cells in association with HLA class II molecules, and that some of these amino acid sequences (HBc_{28-47}, HBc_{50-69}, $HBc_{111-125}$) could associate with multiple HLA class II determinants (Table 2).

Cytokine Profile of T-Cell Lines and Clones. Different experimental strategies were used to analyze the cytokine pattern secreted by HBV-specific T-cell clones. Cytokine production of 26 clones was tested on antigen (or peptide) stimulation in the presence of autologous irradiated APC. Supernatants for cytokine analysis were also obtained by PHA stimulation from 53 clones. Finally, cytokine production of 11 clones was also tested on anti-CD3 and phorbol myristate acetate stimulation.

Irrespective of the type of stimulation protocol, all clones were found to secrete IFN-γ (Fig. 1A), and most of them showed a Th1 cytokine profile, being able to secrete IFN-γ but neither IL-4 nor IL-5. Indeed, 73% and 27% of the T-cell clones stimulated with PHA and 77% and 23% of those stimulated with antigen were Th1 and Th0, respectively (Fig. 2A), whereas no Th2 clones could be identified with either type of stimulation. Eleven clones tested in parallel with PHA or with phorbol myristate acetate plus anti-CD3 showed identical cytokine secretion, with the exception of 2 that showed a Th1 profile on PHA stimulation and a Th0 profile upon anti-CD3 and phorbol myristate acetate activation (data not shown).

The failure to detect Th2 clones in the population of HBV-specific T cells was not caused by the experimental conditions of cloning, because 24% and 18% of tetanus toxoid-specific T-cell clones derived with the same cloning technique from PBMC of two patients with acute hepatitis showed a Th2 profile of cytokine secretion (Fig. 2C and 2D).

The results obtained at the clonal level were confirmed with polyclonal HBV nucleocapsid-specific T-cell lines. Ten of the 15 T-cell lines derived from the acute phase of infection (67%) secreted significant amounts of IFN-γ, but neither IL-4 nor IL-5 (Th1 profile), whereas the remaining 5 lines (33%) produced IFN-γ in association with IL-4 and/or IL-5 (Th0 profile) (Figs. 1B and 2B).

An opposite behavior was observed with the T-cell lines derived from PBMC obtained in the recovery phase (several years following resolution of infection), because 74% of them were Th0, 21% were Th1, and 5% were Th2 (Table 3).

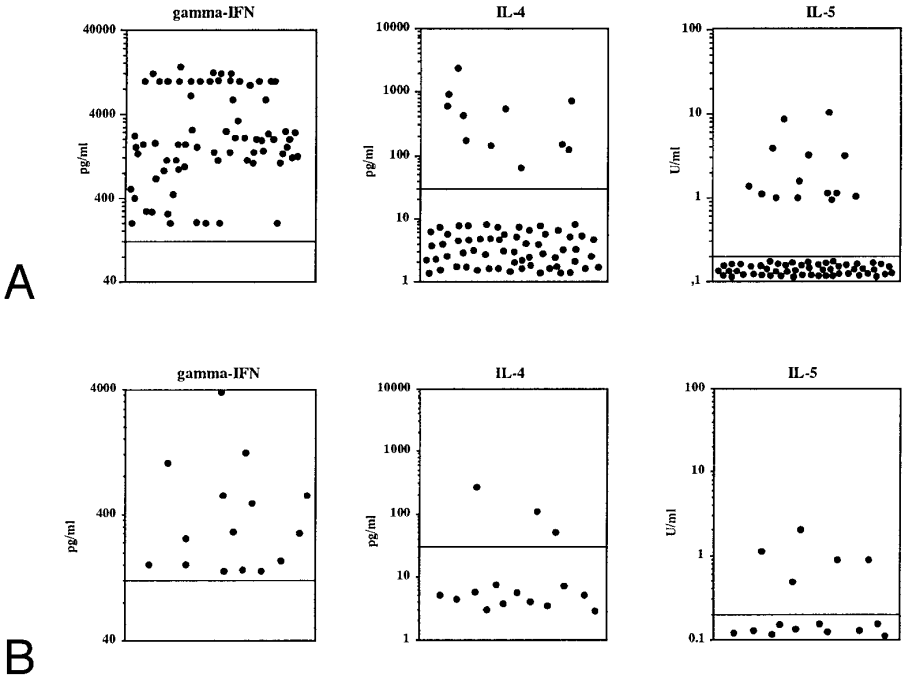

FIG. 1. Levels of cytokines secreted by HBV nucleocapsid-specific T-cell clones (A) and lines (B) derived from 11 patients with self-limited hepatitis B during the acute phase of disease.

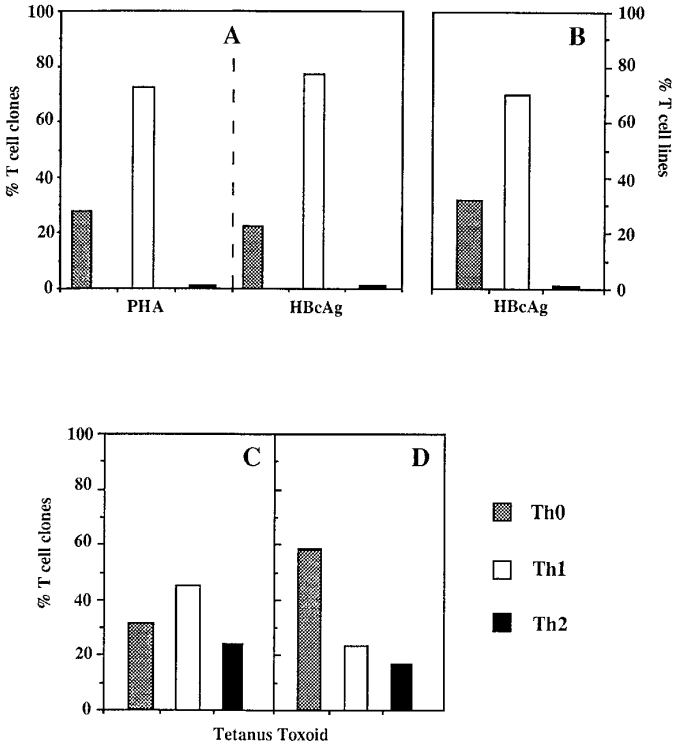

FIG. 2. Cytokine profile of 71 HBV nucleocapsid-specific T-cell clones (A) and 15 lines (B) (upon antigen or PHA stimulation) derived from 11 patients with acute HBV infection, and of tetanus toxoid–specific T-cell clones (upon antigen stimulation) derived from PBMC of two patients (51 clones [C] and 44 clones [D]).

The analysis of the cytokine secretion induced in T-cell lines and clones generated during the acute infection by individual peptides showed a preferential stimulation of Th1-type cytokines by most of the HBV core epitopes, although definitive conclusions cannot be drawn for all peptides, owing to the limited number of clones specific for some of the epitopes (Table 4).

DISCUSSION

Because the type of cytokines secreted by T cells upon recognition of viral antigens is believed to influence both the type of damage caused by the infecting agent and the final outcome of the infection, HBV-specific T-cell lines and clones were produced from the peripheral blood of patients with acute infection to characterize the cytokine profile associated with self-limited hepatitis. T-cell clones and lines were selected by PBMC stimulation with nucleocapsid antigen because it is the most powerful immunogen for HLA class II–restricted T cells in the acute phase of hepatitis B and can activate a strong T-cell response that appears to be temporally associated with the clearance of HBV envelope antigens from the serum.[6,7] The study was performed on the peripheral blood compartment because liver tissue required for purification of lesional T cells cannot be obtained for ethical reasons in patients with acute hepatitis B.

All T-cell clones and lines showed a $CD4^+$ phenotype, and those tested for HLA restriction showed recognition of the stimulatory antigen in association with HLA class II molecules. Some core peptides were presented to T cells by more than one HLA class II allele. This is in keeping with the observation that some peptides were recognized by patients expressing different HLA haplotypes (not shown) and with previous reports indicating that HLA class II–restricted T-cell epitopes can be "promiscuous," i.e., able to activate T cells in the context of different HLA class II alleles.[21-23] Irrespective of the strategy used to stimulate cytokine production *in vitro,* most T-cell lines and clones (approximately 70%) showed a Th1 profile dominated by the secretion of IFN-γ; about 30% were Th0-like cells, able to produce not only IFN-γ but also IL-4 and/or IL-5, whereas no T-cell line or clone showed a polarized Th2 profile. This lack of detection of Th2-type cells among circulating HBV nucleocapsid-specific T lymphocytes in patients with acute HBV infection was not caused by the experimental approach used for cloning, because Th2-type T-cell clones were generated by the same protocol, using a different antigen, such as tetanus toxoid, to stimulate PBMC. Moreover, a prevalent Th0 pattern was observed after recovery from hepatitis.

Because Th1 cells and their cytokines are involved in delayed hypersensitivity, which is a primary defense against intracellular pathogens,[9] the prevalent Th1 pattern of secreted cytokines can be regarded not only as a mechanism contributing to inflammation and local tissue damage, but also as an appropriate response of the immune system to create conditions that hamper viral replication and eventually lead to HBV eradication. Indeed, enhancement of the antiviral activity of macrophages, in particular by stimulation of nitric oxide production,[24-26] induction of resistance to virus in neighboring cells,[27] and increased expression of HLA molecules on infected cells[27] are only some of the antiviral effects of Th1 cytokines. Moreover, in a transgenic mouse model of HBV replication, Th1 cytokines secreted by adoptively transferred cytotoxic T lymphocytes have been shown to down-regulate viral gene expression, leading to HBV clearance from liver cells without their lysis.[28,29] The predominance of a Th1-type T-cell response found at the clonal level in the acute phase of hepatitis B might be related to the production of IL-12 in the liver microenvironment by activated phagocytes involved in the clearance of cell debris derived from dying hepatocytes. Indeed, IL-12 is produced by both macrophages and dendritic cells (reviewed in Trinchieri[30]), and its production elicited by phagocyte-activating stimuli has been found to play a central role in the polarization of Th cells into a Th1-like profile.[31-33]

On the other hand, a cytokine balance in favor of Th2-type responses has been associated with progressive disease by intracellular pathogens, and a shift from Th1 to Th0 or Th2 profiles has been observed in human immunodeficiency virus–infected patients progressing to acquired immune deficiency syndrome.[34,35] Unfortunately, a similar prospective study of the cytokine pattern from the acute to the chronic stage of HBV infection is almost impossible in patients with hepatitis B, because clinically overt, acute HBV infections are generally self-limited and chronic evolution more commonly follows anicteric, clinically silent infections. Furthermore, patients with a long-lasting condition of chronic HBV infection are generally hyporesponsive to HBV antigens at the T-cell level in the peripheral blood compartment, in part as a consequence of the low frequency of circulating HBV-specific T cells.[36] This makes their isolation and the comparison of their functional features with those of T cells from the acute stage of infection a very difficult task. Unfortunately, available information about the cytokine secretion by liver-infiltrating

TABLE 3. Comparison of the Cytokine Profiles of 15 T-Cell Lines Derived From 11 Patients During the Acute Phase of Hepatitis B and 19 Lines Obtained From 6 Subjects Recovered From Acute Hepatitis

Cytokine Profile	Acute Hepatitis (%)	Resolved Hepatitis (%)
Th1	67	21
Th0	33	74
Th2	0	5

TABLE 4. Cytokine Profile of 15 T-Cell Lines and 71 T-Cell Clones (From the Acute Phase of Hepatitis B) in Relation to Their Fine Specificity

	Specificity									
	Amino Acid									
	1-20 (n = 2)	20-34 (n = 14)	28-47 (n = 6)	50-69 (n = 16)	91-110 (n = 1)	100-119 (n = 29)	111-125 (n = 1)	117-131 (n = 3)	120-139 (n = 1)	HBe/c (n = 13)
Th1	2	10	3	13	0	25	0	2	1	7
Th0	0	4	3	3	1	4	1	1	0	6
Th2	0	0	0	0	0	0	0	0	0	0

NOTE. The fine specificity was not defined for 10 clones and 3 lines, indicated as HBe/c-specific (far-right column).

HBV-specific T cells in chronic hepatitis B is limited, because few patients and clones have been studied so far.[37,38] These reports suggest that Th1 cells are enriched within the virus-specific fraction of the liver infiltrates. Moreover, serological studies are consistent with the possibility that patients with chronic active hepatitis B express Th1-like or combined Th1/Th2-like responses.[39] The latter interpretation is in agreement with a recent analysis of the cytokine pattern of the predominant virus nonspecific T-cell population in the liver infiltrates, which indicates that Th0 cells, able to secrete both Th1 and Th2 cytokines, are prevalent within HBV chronically infected livers.[40] Therefore, production of Th1 cytokines in chronic active hepatitis B may contribute to maintain chronic liver damage through delayed-type hypersensitivity reactions. However, the overall Th1 effect might be insufficient to cause complete resolution of infection if the liver environment in chronic hepatitis B is critically influenced by the concomitant effects of Th2 cytokines that might counteract the antiviral protective function of Th1 cells. This is consistent with data in murine herpes simplex virus–mediated stromal keratitis in which Th2 cells exert a pathogenic effect.[41]

In contrast to the extremely low frequency of circulating HBV-specific T cells in chronic hepatitis B, HBV nucleocapsid antigen–responsive T cells are still present at high frequency in the peripheral blood even decades after complete recovery from acute hepatitis B.[36] However, their cytokine pattern seems to differ from that observed in the acute stage of infection, because it is dominated by Th0 cells, able to produce not only IFN-γ but also IL-4 and IL-5. This shift toward a mixed pattern of Th1 and Th2 cytokine secretion may represent a mechanism that evolved to attenuate some effects of Th1-type cytokines (which may become redundant and even harmful to the host after resolution of infection), or simply as a result from switching off IL-12 production following viral clearance and tissue repair. In view of the reciprocal cross-regulation between Th1 and Th2 cytokines,[42] another effect of Th2-type cytokines might be the progressive inhibition of the expansion of the antiviral effector T-cell population once viral clearance has been achieved.

In conclusion, our data are in agreement with the general view that predominant Th1-type T-cell responses can sustain inflammatory reactions, and that Th1-mediated effects at the site of infection can contribute to the local immunopathology. Th1 responses have also been reported to be associated with the capacity of the host to resolve or control viral infections. Therefore, a vigorous T-cell–mediated response with predominant production of Th1-type cytokines may be crucial for successful resolution of hepatitis B. Whether or not only the intensity or also the quality of the T-cell responses are different in patients who succeed in clearing the virus spontaneously compared with those who do not remains to be determined. Lacking this information, our results do not permit definitive conclusions about the role played by Th1 responses in protection against HBV.

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