HUMAN T CELL RESPONSE TO THE SURFACE ANTIGEN OF HEPATITIS B VIRUS (HBsAg)

Endosomal and Nonendosomal Processing Pathways are Accessible to Both Endogenous and Exogenous Antigen

By YIDE JIN, J. WAI-KUO SHIH, AND IRA BERKOWER

From the Center for Biologics Evaluation and Research, Food and Drug Administration and Department of Transfusion Medicine, National Institutes of Health, Bethesda, Maryland 20892

T cells can respond to foreign protein antigens only after they are processed in and presented on the surface of an APC in association with the appropriate MHC molecules (1). Since they only see processed fragments, T cells are generally unable to distinguish between native and denatured proteins (2). It is also possible to replace protein antigens with synthetic peptides that contain the complete antigenic determinant recognized by T cells (3-6). In effect, antigen processing reduces protein antigens to a lowest common denominator recognizable by T cells (7, 8).

At least two distinct pathways for antigen processing have been described. The endosomal processing pathway was described first (9-13) and is distinguished by its sensitivity to inhibitors that raise endosomal pH, such as chloroquine and ammonium chloride, or that compete for the protease binding sites, such as leupeptin (13, 14). In general, endosomal enzymes process exogenous antigens after phagocytosis by the APC (1, 9-13, 15). Presumably, this would be a major pathway by which vaccine antigens could elicit T cell immunity. Recently, a second processing pathway has been recognized, by which nonendosomal proteases produce a different set of antigenic fragments for antigen presentation (5, 15-17). Up to now, this pathway was thought to be accessible only to endogenous viral antigens produced by infected cells. However, if these two pathways are mutually exclusive, it would be difficult for exogenous vaccine antigens to elicit protective T cell immunity against infected cells expressing viral antigens endogenously.

In this paper we demonstrate that cloned human T cell lines, each specific for a single determinant on the surface antigen of hepatitis B virus (HBsAg)[1], can respond to exogenous as well as endogenous antigen. Exogenous antigen was used in the immunization and during the growth and selection of the T cell clones in vitro. Nevertheless, each T cell line responded equally well to infected cells expressing HBsAg endogenously as to exogenous antigen presented by the same APCs. In addition, the epitope specificity and antigen processing requirements for each T cell clone were the same for both endogenous and exogenous antigen. Different T cell clones depended exclusively on endosomal or nonendosomal processing: for our T cells, the antigenic peptides generated by each processing pathway could not substi-

[1] *Abbreviations used in this paper:* HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

tute for the peptide products of the other pathway. Either processing pathway could handle the antigen, regardless of whether it came from an exogenous or endogenous source. These results reveal an underlying symmetry between exogenous and endogenous antigen, which may help explain how T cells elicited by a noninfectious component vaccine can play an active role in the immune response to a subsequent infection with live virus.

Materials and Methods

Antigens. HBsAg was purified from the plasma of a chronic carrier by Sepharose 6B chromatography followed by affinity chromatography as described previously (18). It is known to contain ~5% pre-S antigens, including pre-S_1 and pre-S_2. Purified recombinant yeast-derived hepatitis antigens were the gift of Dr. Ronald Ellis (Merck, Sharp and Dohme Research Laboratories, West Point, PA) and included: S antigen, Pre-S_1, Pre-S_2, and long S (pre-S_1 + pre-S_2 + S) (19). Synthetic peptides 21-47 of the pre-S_1 region and 120-145 of the pre-S_2 region were produced on an Applied Biosystems solid phase peptide synthesizer and were a gift of Drs. Frank Robey and Mei Ying Yu (Office of Biologics Research and Review, FDA). Pre-S_1 peptide 1-28 was a gift of Dr. John Gerin (Georgetown University, Washington, DC). Pre-S_1 peptide 12-32 was a gift of Dr. Brad Jameson (California Inst. of Technology, Pasadena, CA), as were the other pre-S_1 peptides.

Monoclonal Antibodies. Monoclonal anti-HLA-ABC was purchased from Cappel Laboratories (Malvern, PA) and was added to culture at 5 μg/ml. Monoclonal anti-HLA-DR clone L243 was purchased from Becton Dickinson & Co. (Mountain View, CA). It was used to pretreat APC overnight at a concentration of 2 μg/ml as described previously (20).

Recombinant Vaccinia Viruses. Vaccinia recombinants expressing HBsAg were a gift of Drs. Bernard Moss, Geoffrey Smith, and Kuo-Chi Chen (National Institute of Allergy and Infectious Diseases, Bethesda, MD). Recombinant v55 expresses the S region only, and the protein product is secreted as 22-nm particles (21). Recombinant v73 expresses the entire pre-S +S region, and the protein product remains cell associated until the cell dies (22, 23). The vaccinia recombinants were lightly UV treated (20 s at 14 cm from a 700-W lamp with a peak wavelength of 254 nm) so they would express early genes (including the hepatitis antigens) without releasing infectious vaccinia virus. Higher doses of UV light gave decreased expression of S antigen, as measured by RIA and a proportionate decrease in T cell stimulation (data not shown). Before infection, the EBV cells were treated with mitomycin c (50 μg/ml) for 40 min, washed three times, and then exposed to the vaccinia recombinant for 1 h at a multiplicity of infection of 10. They were then washed to remove excess free virus.

Subjects. Two individuals lacking antibodies to hepatitis B surface and core antigens received three doses of plasma-derived (donor LL) or recombinant (donor SR) vaccine between 1 and 2 yr before these studies. Donors of APCs were HLA typed at the NIH Clinical Center HLA laboratory.

Proliferation Assay. The T cell proliferation assay was performed as described previously (24). PBL were separated from heparinized blood by sedimentation onto a layer of Ficoll-Hypaque. These cells were then depleted of adherent cells by incubating on plastic for 3 h at 37°C. The nonadherent cells were added to culture at 4×10^5 per microwell with an additional 10^5 irradiated PBL (3,300 rad) as a source of APCs. Plasma-derived HBsAg or other antigens were added at 2-4 μg/ml. After 4 d in culture, 1 μCi of [^{3}H]thymidine was added for an additional 18 h and labeled cells were harvested on an automated device. Incorporation of [^{3}H]thymidine into new DNA was measured in a liquid scintillation counter. Results are expressed as the geometric means of triplicate wells minus the medium control (Δ cpm). The medium control value is given in parentheses, and the SEM was generally within 10% and is not shown.

Establishing a T Cell Line and Cloning by Limiting Dilution. The derivation and initial characterization of the cloned LL1 line from donor LL was described previously (25). PBL from donor SR were found to give a good proliferative response to HBsAg, so 10 microwells were started in culture as described for the proliferation assay, except that the cells were harvested

on day 7 and cloned by limiting dilution at 1 or 0.3 cells per well. Cloning conditions included 2×10^4 irradiated PBL feeder cells in round-bottomed wells, containing 50% RPMI 1640 and 50% EHAA media supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, 2 mM glutamine, 5×10^{-5} M mercaptoethanol, 10% autologous serum, and 10% IL-2 (Cellular Products, Buffalo, NY). After 2-3 wk, proliferating cells were apparent in 67 of 480 wells. This cloning efficiency is typical of our results with human T cell clones. Of these, 24 were antigen specific, including 3 from the plates receiving 0.3 cells per well. The cloned lines were propagated by repeated cycles of stimulation in microwells followed by expansion in macrowells in the presence of 10% IL-2. Two of the SR clones (SR45 and SR54) were used in the present studies, because their HLA restriction and antigen processing requirements were found to contrast with those of LL1.

Results

Human T Cell Lines Specific for HBsAg. Vaccination with plasma-derived or recombinant HBsAg elicits a detectable T cell response in a small proportion of people tested (26). As reported previously (25), we tested 20 vaccinees and found 3 individuals whose T cells gave a significant in vitro proliferative response to exogenous HBsAg. For each responder, the population of antigen-specific T cells was expanded by antigen stimulation, and then cloned by the limiting dilution method. We obtained a T cell line LL1 from one donor and T cell clones SR45 and SR54 from a second donor. Each of these clones was characterized for HLA restriction, epitope specificity, ability to recognize endogenous antigen, and antigen processing requirements for both endogenous and exogenous antigen.

HLA Restriction. T cell line LL1 responds to HBsAg only when the HLA antigens of the APC match the HLA of the donor. We have determined which MHC antigen was recognized as a restriction element by LL1 by studying PBL from family members JL and JoL for the ability to present antigen to the line. As shown in Table I, Exp. 1, PBL from JL gave maximal antigen-specific stimulation, while PBL from JoL and the unrelated IB gave none at all. Comparing the HLA types of each donor, we observed that HLA-A1, B35, and C4 were present on stimulatory and nonstimulatory presenting cells alike. HLA-A11 was present only on stimulatory PBL and could explain the observed pattern of stimulation. In other experiments, PBL from four of five unrelated HLA-A11 donors gave maximal stimulation by HBsAg (Table I, Exp. 2, shows three of these). Thus, the LL1 line recognizes HBsAg in association with HLA-A11.

In contrast, the two clones SR45 and SR54 appeared to recognize HBsAg in association with HLA-DR7. The donor SR typed as HLA-DR6,7, and the clones respond to APCs bearing HLA-DR7 but not DR6 (as in Table IV and Fig. 2 below). mAbs to MHC class I or class II antigens were then tested for the ability to block the antigen-specific response of each clone (Table II). mAbs to HLA-A,B,C completely inhibited the LL1 response to plasma-derived HBsAg, while anti-HLA-DR had little effect. Conversely, the monoclonal anti-HLA-DR inhibited the SR45 response to yeast-derived HBsAg, while anti-HLA-ABC inhibited poorly. Interestingly, anti-HLA-DR also completely inhibited the response of SR54 to endogenously expressed HBsAg (discussed further below), while anti-HLA-ABC was less effective. Thus, LL1 responds to HBsAg in association with the MHC class I determinant HLA-A11, while SR45 and SR54 are specific for MHC class II.

Epitope Mapping. As shown in Table III *A*, yeast recombinant polypeptides de-

TABLE I
MHC Restriction of the LL1 T Cell Line

Exp.	APC donor	Cells	HLA A	HLA B	HLA C	HLA DR	[^{3}H]TdR incorporation No Ag	[^{3}H]TdR incorporation + HBsAg
							Δ cpm	
1	LL	PBL	1/11	8/35	4	7	1,227	145,086
	JL	PBL	1/11	35/35	4	4	1,508	134,719
Family members		EBV					1,709	61,497
	JoL	PBL	1/1	8/35	4	7	447	899
		EBV					606	668
	IB	PBL	2,3	18,35	4,6	ND	330	2,642
2	LL	PBL	1,11	8,35	4	7	(1,303)	19,610
	BL	PBL	11,23	14,17	5	6,8	−558	15,635
Unrelated donors	TS	PBL	11,33	14,62	4	1,8	−10	24,376
	DB	PBL	11,31	51,35	4	ND	808	12,674
	WF	PBL	1	8	6	3	460	314
	IB	PBL	2,3	18,35	4,6	ND	339	131

MHC restriction of the LL1 T cell line on family members' PBL in Exp. 1 or on PBL from unrelated donors in Exp. 2. JL antigen-presenting cells, which stimulate the clone in the presence of HBsAg, share four HLA class I antigens with the propositus LL. However, of these only HLA-A11 is lacking in the JoL cells that do not stimulate, suggesting that HLA-A11 is the class I restriction element.

rived from different regions of HBsAg were tested for the ability to stimulate each T cell line. LL1 was stimulated by long S (containing pre-S_1 + pre-S_2 + S) and by pre-S_1 alone, but not by S antigen. Conversely, SR54 was stimulated by long S and by S antigen alone, but not by pre-S_1. SR45 was also stimulated by recombinant S antigen alone which is how it was derived. Thus, LL1 recognizes an epitope in the pre-S_1 region, while SR54 and SR45 respond to epitopes in the S region of HBsAg.

Further precision in mapping the pre-S_1 epitope recognized by LL1 required the

TABLE II
MHC Class I or II Restriction of T Cell Lines

mAb	T cell line LL1*	SR45*	SR54‡
None	61,133	101,269	73,435
Anti-ABC	−486	64,721	22,185
Anti-DR	47,975	15,793	990
Medium control	(799)	(1,588)	(837)

The same treated or untreated EBV-derived B cells (from donor LL) were used as the APC for all three T cell clones. For clone SR54, the EBV line was infected with vaccinia recombinant v73 as described in Materials and Methods. The APC were either pretreated overnight with monocloanl anti-HLA-DR (2 μg/ml), or cultured in the presence of anti-HLA-ABC (5 μg/ml). Data are expressed as Δ cpm, with the medium controls in parentheses.

* LL-EBV as APC with exogenous HBsAg (2 μg/ml).

‡ v73-infected LL-EBV expressing endogenous HBsAg.

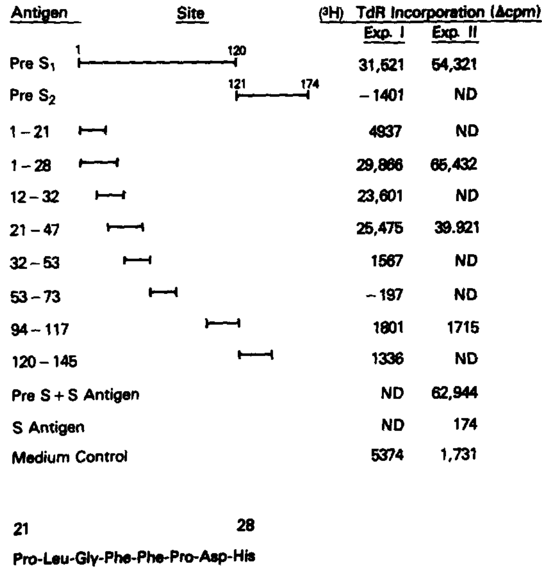
TABLE III
Fine Specificity of Human T Cell Lines for HBsAg

A. Exogenous recombinant antigen		[^{3}H]TdR incorporation	
APC	Antigen	LL1	SR54
		Δ cpm	
B cell line	$PreS_1 + PreS_2 + S$	26,450*	13,865‡
B cell line	$PreS_1$	37,460	600
B cell line	S	2,587	68,457
B cell line	Medium control	(2,176)	(1,588)
B. Endogenously expressed antigen			
Stimulator cell infected by	Antigen expressed		
v73	$PreS_1 + PreS_2 + S$	44,803*	76,106‡
v55	S	−865	47,008
Vaccinia wild type	–	−1,383	1,112
Uninfected control	–	1,911	2,094

Each T cell line was stimulated with exogenous or endogenous HBsAg polypeptides from the pre-S or S region. In *A*, each exogenous antigen was added at 2 μg/ml with HLA-matched EBV-derived B cells as the APC. In *B*, the same B cells were infected with v55 (expressing S antigen) or v73 (expressing pre-S + S antigen), at a multiplicity of infection of 10, incubated for 1 h at 37°, washed, and added to culture as infected stimulator cells expressing endogenous HBsAg.
* For the LL1 T cell line, the haploidentical JL-EBV B cell line was used.
‡ For the SR54 T cell line, the D_R-matched 898-EBV B cell line was used.

use of synthetic peptides. Recent work with cloned T cell lines specific for a variety of antigens has shown that complete epitopes recognized by T cells may be contained on synthetic peptides corresponding to the antigenic determinant on the protein (reviewed in references 8 and 27). Thus, we tested an overlapping series of synthetic peptides corresponding to the pre-S_1 sequence for the ability to stimulate LL1. As shown in Fig. 1, the response to recombinant yeast-derived pre-S_1 was equal to the response to the entire pre-S_1 + pre-S_2 + S antigen. In addition, three of the synthetic pre-S_1 peptides gave maximal stimulation, indicating that the entire antigenic determinant recognized by LL1 is contained on these peptides. The three peptides 1–28, 12–32, and 21–47 overlap at residues 21–28. We conclude that the epitope recognized by LL1 is located in this region and has the sequence Pro-Leu-Gly-Phe-Phe-Pro-Asp-His (28).

Recognition of Endogenous HBsAg. An important question for vaccine-immune T cells is whether they can respond to infected cells that express viral antigens endogenously. To get endogenous expression of HBsAg in lymphocytes, we infected the cells with vaccinia recombinants that were constructed with either the S region only (v55) or with the entire long S region (v73), including pre-S_1 + pre-S_2 + S. Previous studies have shown that cells infected with v55 produce S antigen and secrete 22-nm particles, while cells infected with v73 produce long S protein but the antigen remains a monomer and is not secreted (22). As shown in Table III *B* and Fig. 2, LL1 responds to v73-infected cells that express pre-S_1 + pre-S_2 + S, but not to v55-infected cells that express S antigen only. SR54 responds well to stimulator cells infected by either recombinant virus, while neither T cell line responds



Antigen	Site	(^{3}H) TdR Incorporation (Δcpm) Exp. I	Exp. II
Pre S_1	1–120	31,621	54,321
Pre S_2	121–174	−1401	ND
1−21		4937	ND
1−28		29,866	65,432
12−32		23,601	ND
21−47		25,475	39.921
32−53		1567	ND
53−73		−197	ND
94−117		1801	1715
120−145		1336	ND
Pre S + S Antigen		ND	62,944
S Antigen		ND	174
Medium Control		5374	1,731

21 28
Pro-Leu-Gly-Phe-Phe-Pro-Asp-His

FIGURE 1. T cell response to synthetic peptides of HBsAg. Epitope mapping with synthetic peptides. LL1 was incubated with each peptide or with recombinant pre-S_1 antigen at a concentration of 0.1 μM, or with recombinant pre-S_2 antigen at 0.3 μM. The identical pattern was obtained with each peptide at 0.5 μM. Three overlapping peptides gave maximal stimulation. They share amino acids 21–28, and the shared sequence is shown.

to cells infected by vaccinia wild type. SR45 also responds to v55, but only when the infected stimulator cell matches at HLA-DR7 (Fig. 2 *C*). The response to endogenous antigen was generally greater than or equal to the response to exogenous antigen for the doses tested. For all three clones, the response to v55-infected cells correlated with whether the clone responded to exogenous S antigen (Table III). The results are compatible with each clone recognizing the same epitope for endogenous as well as exogenous antigen.

The demonstration that the T cells are responding to endogenous antigen depends on the assumptions that (*a*) exogenous HBsAg does not contaminate the recombinant virus and (*b*) HBsAg is not secreted by an infected cell and taken up and processed as exogenous antigen by a second cell. This was supported by four lines of evidence:

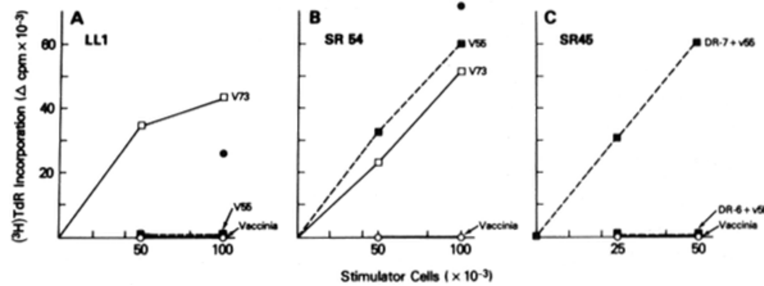


FIGURE 2. T cell response to infected stimulator cells. Each T cell line was incubated in the presence of increasing numbers of HLA-matched EBV-derived B cells that were either uninfected, or infected with vaccinia wild type (no HBsAg), v55 (expressing S antigen), or v73 (expressing pre-S + S antigen). With uninfected APCs, the response to exogenous antigen is shown as a closed circle, and the background for LL1 was 141 cpm, 1,522 cpm for SR54, and 802 cpm for SR45 (which increased to 92,080 cpm in the presence of HBsAg).

TABLE IV
Mixing Experiment

Infected cell	Uninfected cell	DR of infected cell	Proliferation of SR54
			Δ cpm
JL-EBV	–	DR7	20,876
1101-EBV	–	DR6	637
JL-EBV	1101-EBV	DR7	11,143
1101-EBV	JL-EBV	DR6	−1,790
	JL-EBV	–	(2,275)
	1101-EBV	–	−835
	JL-EBV + HBsAg	–	33,344

Mixing experiment to detect secretion and reuptake of endogenously expressed HBsAg. Infected 1101 B cells of the irrelevant HLA type (DR-6) were tested alone or in the presence of uninfected JL B cells of the relevant HLA type (DR-7) for the ability to stimulate SR54. If the v73-infected cells secreted HBsAg, then it could be taken up and presented by the uninfected JL B cells.

First, there was little or no detectable exogenous HBsAg associated with the pure virus, as measured by RIA (data not shown). Second, HBsAg production was under the control of viral genes: excessive UV irradiation of v55 (3–30-fold greater than the optimal dose) gave an exponential decrease in antigen production and an equal decrease in T cell stimulation (data not shown). Third, v73 is known to express endogenous pre-S_1 + pre-S_2 + S, but this long S antigen is not secreted into the culture medium, as shown by Western blots (22). Fourth, we tested whether the T cell response to v73-infected cells could be due to reuptake of secreted antigen (Table IV). We mixed infected 1101 cells of the mismatched HLA type (DR-6) with uninfected JL cells of the matched HLA type (DR-7). The JL cells were competent presenters of exogenous antigen as shown in the last line, and of endogenous antigen as shown in the first line of the table. However, when mixed with infected 1101 cells they were unable to take up enough antigen to stimulate clone SR54. Thus, T cells recognize the endogenous antigen of infected cells directly, without resorting to a second APC.

Distinct Antigen Processing Pathways. Previous studies have shown that T cells commonly recognize the same antigenic determinant whether contained on a native protein or a synthetic peptide (8, 27). Synthetic peptides can also substitute for endogenously expressed viral antigens (5, 16, 17). In both cases, the peptides are fully competent to stimulate a response, suggesting that partial proteolytic degradation is important for the presentation of native protein antigens from an endogenous or exogenous source. In addition, the steps of antigen processing have been divided into two distinct pathways based on sensitivity to inhibitors of endosomal proteases. The endosomal pathway has frequently been implicated in the processing of exogenous native proteins for MHC class II–associated antigen presentation. It is readily inhibited by chloroquine or leupeptin (9–13). The nonendosomal pathway is thought to process endogenously expressed viral proteins primarily, since CTL recognize infected target cells despite pretreatment with chloroquine, and also recognize uninfected targets when pulsed with synthetic peptides corresponding to viral antigens but not with the native protein antigens (5, 17). Thus, an alternative, nonendosomal pro-

TABLE V

Antigen Processing Requirements for Exogenous and Endogenous HBsAg

T cell clone	Stimulus	$[^3H]$TdR Incorporation	
		Alone	+ Leupeptin (1 mM)
LL1	Exogenous HBsAg	25,932	43,339
	v73 infection	34,829	64,446
SR54	Exogenous HBsAg	71,042	1,486
	v73 infection	23,134	1,902

For LL1, the autologous EBV-derived B cell line was used either as APCs or as v73-infected stimulator cells in the presence or absence of 1 mM leupeptin. The background response with uninfected B cells was 141 cpm and with the vaccinia infected control cells was 17 cpm above background. For SR54, an HLA-DR7-matched B cell line (898) was used in the same way, giving a background of 1,522 cpm with uninfected cells and a vaccinia control of 21 cpm below background. SR54 requires endosomal processing (leupeptin-sensitive) of both endogenous and exogenous HBsAg, while LL1 does not require the leupeptin-sensitive processing step for either source of antigen.

cessing pathway was postulated to work primarily on endogenous viral antigens, while the endosomal processing pathway was thought to handle exogenous antigens (5, 15, 17).

Since our T cell lines recognize both endogenous and exogenous HBsAg, we could test whether there is a strict segregation of processing pathways, or whether each processing pathway is accessible to both internal and external antigen. An example of such an experiment is shown in Table V. The class I-restricted LL1 response to exogenous HBsAg is totally resistant to leupeptin inhibition, and so is the response to endogenous HBsAg produced by v73 infection. This suggests that the nonendosomal processing pathway is readily accessible to both exogenous and endogenous antigen. In contrast, the class II-restricted SR54 response to HBsAg is totally inhibited by leupeptin, regardless of whether the antigen comes from outside or inside the cell. Thus, the endosomal pathway is also accessible to both forms of antigen. The complete shutdown of the lysosomal pathway indicated by the inhibition of the

TABLE VI

Chloroquine Effect on the Response to Endogenous HBsAg

T cell line	Stimulator cell infected by	$[^3H]$Thymidine incorporation	
		Alone	+ Chloroquine (100 μM)
		Δcpm	
LL1	v73	122,042	96,051
SR54	v73	88,766	4,990

Chloroquine inhibits the SR54 response but not the LL1 response to endogenous HBsAg. EBV-derived B cells from a single donor who was histocompatible to both LL and SR were infected with v73 for 1 h, followed by 1-h treatment with nothing or 0.1 mM chloroquine. They were washed and added to culture as stimulator cells. V73-infected cells stimulated LL1 despite chloroquine treatment, while the SR54 response to the same cells was 94% inhibited. The LL1 background with uninfected B cells was 3,212 cpm, and the SR54 background was 1,451 cpm.

SR54 response has no effect on the response of LL1, other than to increase it by 65% or more. Thus, the two processing pathways are independent, and the products of one pathway cannot substitute for the other in stimulation of T cells.

As shown in Table VI, we repeated this experiment with chloroquine as the inhibitor of endosomal proteases. However, we could only test the effect of chloroquine on endogenous antigen, since we were unable to pulse the APCs with exogenous antigen. Once again, full inhibition of the endosomal pathway was indicated by the 94% inhibition of SR54, but LL1 continued to respond to the antigen maximally. Thus, endogenous antigen can stimulate SR54 via a chloroquine sensitive pathway, or it can stimulate LL1 via a chloroquine insensitive pathway. The results in these two tables suggest that two distinct pathways exist for processing antigens that will eventually stimulate T cells like LL1 or SR54. Each T cell sees the product of one pathway only. But both pathways are accessible to endogenous and exogenous antigens.

Discussion

We have found that plasma- or yeast-derived hepatitis B vaccine can elicit human T cells immune to the pre-S or S regions of HBsAg. They recognize HBsAg in the context of MHC class I in some cases or class II antigens in other cases. Each cloned T cell line responds to endogenously expressed viral antigen as well as to exogenous antigen. Although each of the T cell lines recognizes processed antigen, more than one proteolytic pathway is available to process viral proteins into antigenic fragments that can be recognized by the T cell lines.

The antigenic determinant recognized by LL1 mapped to the pre-S_1 region, based on the response to recombinant pre-S_1 and long S and the failure to respond to S alone. More precise epitope mapping was based on synthetic pre-S_1 peptides 1–28 and 21–47, which stimulate the line (as does 12–32), while other pre-S_1 peptides do not. The antigenic determinant consists of amino acids 21–28, which has the sequence Pro-Leu-Gly-Phe-Phe-Pro-Asp-His (28). Presumably, this peptide contains distinct functional sites for MHC association (with HLA-A11) and for binding by the antigen receptor of LL1 (29–32). This site is close to but distinct from the pre-S_1 site 12–21 identified by Milich et al. (33) as the pre-S_1 antigenic determinant recognized by mouse T cells in association with MHC class II antigens. They found that T cells specific for this site could provide T helper function to B cells specific for the S region (33). The LL1 T cell line is different in that it responds to peptide 21–28 in association with MHC class I antigens, and we do not as yet know whether it provides helper function.

The sequence of 21–28 fits well into the paradigm of Gly-hydrophobic-hydrophobic-Pro-charged amino acid proposed by Rothbard et al. (27, 34). The closest reported analogue is peptide 111–120 of influenza hemagglutinin, which is presented in association with class II antigens in the mouse (35). It is not clear whether peptides that associate with MHC class I antigens will follow the same or a different pattern from those that associate with class II antigens (7, 8). However, in both cases, we presume that antigen processing is required because peptides can bind to MHC molecules but native proteins cannot (13, 30–32). LL1 responds well to synthetic peptides, and we presume that the independence of endosomal processing reflects use of a nonendosomal processing pathway, rather than the absence of processing.

Nonendosomal processing pathways are found in a large variety of cells (36). Or-

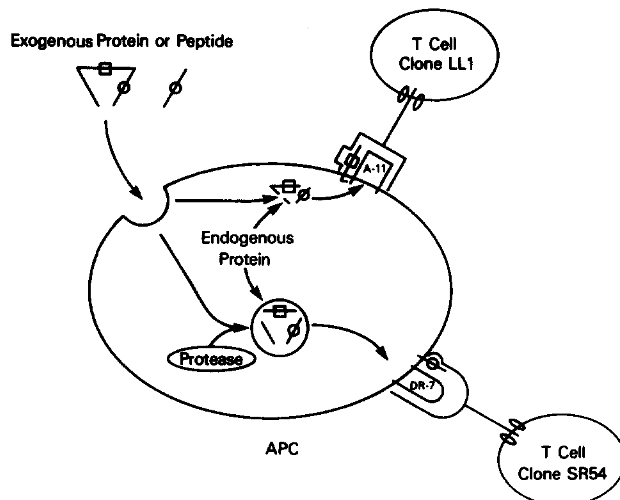


FIGURE 3. Schematic of antigen processing. Endosomal processing (in the circle) and nonendosomal processing pathways are accessible to both exogenous and endogenous antigen.

dinarily, they are important for the turnover of used or defective cellular proteins. But, in the infected cell, they may provide an important immunological signal of viral infection in the form of antigenic peptides on the cell surface in association with MHC antigens. Further progress may depend on finding selective inhibitors of the nonendosomal pathway, so that its unique physiological role can be determined.

Our results on the processing of HBsAg for T cell stimulation are summarized in Fig. 3. Exogenous antigen is endocytosed by the APC and partially degraded into antigenic fragments. Endogenously expressed antigen is processed in a similar manner. The processed peptides associate with MHC antigens on the surface of the APC. Receptor binding triggers the T cell response, including proliferation and IL-2 production. It does not matter to the T cell whether the antigen comes into the culture as a native protein or a peptide, since only peptides are able to associate with MHC and stimulate T cells. The model shows two possible processing pathways: one is endosomal and would be inhibited by leupeptin or chloroquine; the other is nonendosomal and insensitive to these inhibitors. As reported by others (5, 15, 37), individual T cells see the products of one pathway or the other, but not both. However, in contrast to those reports, we find that exogenous antigen is not limited to the endosomal pathway, but also can be processed via the nonendosomal pathway (Table V). Inhibition of the endosomal pathway has no effect on nonendosomal processing of exogenous antigen, suggesting that the two pathways are independent and operate in parallel with each other. Secondly, we find that endogenous antigen is not limited to the nonendosomal pathway, but can also be processed via the endosomal pathway (Tables V and VI). Peptides produced from endogenous proteins show the same antigenicity (Table III and Fig. 2) and the same sensitivity to endosomal inhibitors as do peptides produced from exogenous proteins.

In light of the fact that all of our exogenous HBsAg-specific T cells crossreact with endogenous antigen, we have tried to understand why none of the influenza nucleoprotein and hemagglutinin-specific clones do so (5, 15). There are two major differences between the types of T cell clones studied. Ours were proliferative clones from

noninfected donors who received a component vaccine. Theirs were cytolytic clones from mice that were acutely infected with live virus. The difference between proliferative and cytolytic T cells seems unlikely to explain a difference in antigen processing, since the TCR is substantially the same for both types of clones (38, 39) so the recognition of peptide-MHC complexes should be similar.

Alternatively, different T cell clones may be elicited by live virus than by vaccination with purified viral components. Fortunately, the vaccine-immune T cells cross-react with both endogenous and exogenous antigens, even though the infection-derived T cells apparently do not.

Each of our three clones (plus two others not shown) was able to recognize endogenously expressed HBsAg as well as exogenous antigen. This is in spite of immunization in vivo with noninfectious vaccine antigen and propagation of the T cell lines in vitro with exogenous antigen. Further, we have tested whether recognition of endogenous HBsAg could be explained as secretion by infected cells followed by reuptake and presentation of exogenous antigen by uninfected APCs. No evidence for this mechanism was detected in the mixing experiment (Table IV). In addition, endogenous pre-S + S antigen expressed by v73 is not secreted but remains cell associated where it is recognized by the T cells. Recognition of endogenous antigen by T cells immune to exogenous HBsAg was a general finding with every one of our T cell lines and applied to both MHC class I- and class II-specific T cells. Thus, a noninfectious viral component vaccine can elicit active T cell immunity against infection with live virus.

Summary

We have studied the antigen specificity and processing requirements of three vaccine-induced cloned human T cell lines specific for HBsAg, the envelope protein of hepatitis B virus. Each T cell line recognized endogenously expressed antigen as well as exogenous antigen. Two clones required endosomal processing, both for exogenous and endogenous antigen; while the other T cell line depended on nonendosomal processing to generate antigenic peptides from both endogenous and exogenous antigen. Thus, the two processing pathways are accessible to exogenous and endogenous antigen. These results suggest that vaccine-induced T cells can participate actively in the immune response to live virus.

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