Ann. Ist. Super. Sanità
Vol. 27, N. 1 (1991), pp. 37-40

IDENTIFICATION OF T CELL EPITOPES ON HEPATITIS B SURFACE ANTIGEN

S. VENTO

Unità Malattie Infettive, Ospedale "A. Pugliese", Catanzaro, Italy

Summary. - *The antigenic sites for human T lymphocytes on hepatitis B surface antigen (HBsAg) have been studied using synthetic peptides. The results indicate that aminoacid residues 24-28 of HBsAg (located near the amino terminus of the HBsAg molecule) constitute an immunodominant "helper" determinant, whereas residues 17-18 and/or 34-36 represent a major "suppressor" epitope. However, non responsiveness to currently used hepatitis B virus (HBV) vaccines (employing the whole HBsAg molecule) does not depend, in the vast majority of cases, on suppressor T cells.* In vivo *experiments with synthetic peptide vaccines are needed to verify the possibility of enhancing the production of protective antibodies against the hepatitis B virus.*

KEY WORDS: T cell epitopes, virus hepatitis B, synthetic peptides.

Riassunto (Identificazione di epitopi T delle proteine di superficie del virus dell'epatite). - *La risposta immunitaria anticorpale verso HBsAg (226 aminoacidi), proteina di superficie del virus dell'epatite B (HBV), è essenziale sia per la clearance virale in corso di epatite acuta che per la protezione dall'infezione. Poiché i linfociti T promuovono e controllano la risposta anti-HBs, l'identificazione dei determinanti, nella molecola di HBsAg, riconosciuti dai linfociti T_H e T_S è di grande rilevanza al fine di influenzare positivamente la risposta anticorpale. I risultati degli esperimenti effettuati indicano che i residui aminoacidici 24-28 di HBsAg rappresentano un determinante immunodominante "helper", mentre i residui 17-18 e/o 34-36 costituiscono un importante epitopo "suppressor". La mancata risposta ai vaccini anti-epatite B attualmente in uso (costituiti dall'intera molecola di HBsAg) non dipende, però, nella grande maggioranza dei casi, dall'attività di cellule T suppressor. Soltanto esperimenti* in vivo *con peptidi sintetici contenenti epitopi immunodominanti "helper" potranno consentire di stabilire con certezza se vaccini peptidici stimolano maggiormente la produzione di anticorpi protettivi contro HBV rispetto agli attuali vaccini e sono, quindi, candidati a sostituirli.*

PAROLE CHIAVE: epitopi T, virus dell'epatite B, peptidi sintetici.

Introduction

The hepatitis B virus (HBV) is a DNA virus, whose main translation products are the nucleoprotein "core" antigen (HBcAg) and the "surface" antigen (HBsAg).

The envelope protein, HBsAg (226 amino acids), plays an essential role in the induction of protective immune responses against the virus; hence, subjects who recover from an acute HBV infection and those responding properly to the HBsAg vaccine mount both cellular and humoral immune responses to HBsAg [1, 2] whereas patients with chronic HBV infection and non responders to the vaccine do not show cellular [3] or humoral [4] immune reactions to HBsAg.

Effector T lymphocyte and antibody responses to HBsAg depend on T_H lymphocytes [5, 6] and the surface antigen of HBV must be recognized by both B and TH cells in order to induce a protective antibody response.

Several epitopes in HBsAg appear to be recognized by antibodies and the determinants called a, d, r and w allow to differentiate among the strains of HBV [7, 8] The "a" determinant is particularly important, as: a) it is present in all known strains of HBV, b) some antibodies inhibit viral infection [9] and c) the majority of the anti-HBs antibodies produced by HBV vaccine recipients are directed to this epitope [10].

In contrast, much less is known about the recognition sites for human T lymphocytes in the HBsAg molecule. Such studies are particularly complex, as, unlike antibodies, T_H cells do not bind the antigen directly but recognize only antigens "presented" on the surface of an antigen presenting cell in association with MHC class II molecules [11] and macromolecules and viral particles must be processed into simpler peptides by the antigen presenting cell before the interaction with the MHC class II molecule can occur.

Despite these difficulties, Milich *et al.* have extensively studied the epitopes on HBsAg recognized by T cells in a murine model. Initially [12] they showed that the response

of T cells to HBsAg varied according to the MHC haplotype of the mouse strain studied. Further experiments with the use of synthetic peptides of HBsAg demonstrated the existence of specific recognition sites for murine T cells [13].

Pivotal experiments in humans have been performed essentially by Celis *et al.* at the Wistar Institute, Philadelphia. Their studies of the cellular immune response to HBsAg have led initially to the isolation of antigen-specific T cell lines and clones from HBV vaccinees. These cells were of the helper/inducer class of T lymphocytes as expressed the cell surface molecule CD4 and were capable of inducing *in vitro* the synthesis of anti-HBs antibodies by antigen-stimulated autologous B lymphocytes [14].

Subsequently the same authors have analyzed the fine specificity of those clones for the HBsAg molecule. In order to do this effectively they have prepared synthetic peptides bearing amino acid sequences of HBsAg and have considered the two general models proposed in recent years to identify the amino acid sequences of antigens involved in T cell recognition.

As everybody knows, while De Lisi and Berzofsky have proposed that T lymphocytes react with α-helical, amphipathic structures contained within the amino acid sequences of the antigen proteins [15, 16], Rothbard, McMichael and Townsend have identified a 4- to 5-amino acid sequence motif in a large number of T cell antigenic determinants [17, 18]. This motif is composed of a glycine or a charged amino acid residue in the first position, followed by 2 or 3 hydrophobic residues, which are followed in turn by either a glycine, a polar or a charged residue in the last position.

By using a computer program, De Lisi and Berzofsky have also determined that amino acid residues 23-39 of the HBsAg molecule have the highest amphipathic score of the whole molecule and are therefore most likely to contain a T cell determinant [16].

Celis *et al.* have prepared 10 synthetic peptides choosing the amino acid sequences from the nucleotide sequences of the S gene of HBV of either the ayw [19] or adr [20] subtypes. The peptides are called S1 (amino acid positions 4-33), S2 (34-53), S3 (57-73), S4 (64-94), S5 (93-112), S6 (113-132), S7 (136-155), S8 (163-192), S9 (202-222), S10 (212-222). The results of their studies indicate that a significant number of human T cells (isolated from various hepatitis B vaccine recipients) which react with HBsAg also recognize the synthetic peptide S1; experiments with smaller and overlapping peptides of this region of HBsAg suggest the existence of only one T cell determinant and indicate that residues 26-28 are critical in determining the T cell epitope [21].

Results

To confirm and extend the above observations, I have used only one of the S1 region smaller peptides prepared by Celis *et al.* (S1b, residues 19-33 of the HBsAg molecule, a kind gift of E. Celis), as its residues have the highest amphipathic score of the whole molecule [16] and it comprises the 4-amino acid sequence motif (Arg-Ile-Leu-Thr, residues 24-27) proposed by Rothbard *et al.* Thus this peptide appears to have all the requisites for T cell recognition.

Since the amino acid residues bordering the Arg-Ile-Leu-Thr sequence also appear to play a significant role in defining the reactivity of the T cells [21], I have also employed a second peptide named "T-cell R"(kindly donated by B. Ramage, Department of Organic Chemistry, University of Edinburgh, United Kingdom), containing residues 17-36 of the HBsAg molecule and thus comprising the S1b peptide.

To determine whether not only cloned T cells from HBV vaccine recipients but also uncloned peripheral blood helper/inducer CD4-positive lymphocytes from both HBV vaccinees and subjects recently recovered from acute HBV infection, recognize S1b and/or "T-cell R" peptides, I have employed the above peptides (at a concentration of 100 μg/ml) and recombinant HBsAg (kindly donated by BIOGEN S.A., Geneva, Switzerland, at a concentration of 10 μg/ml) in an indirect T lymphocyte ingression migration inhibitory factor (T-LIF) assay [1, 3, 22].

The results [23] have confirmed that helper inducer T cells reactive to HBsAg are present in the circulation in subjects recovered from acute hepatitis B and in "responder" vaccinees (vaccine: HEVAC 8, Pasteur Institute, Paris), whereas chronic carriers and "non responder" vaccinees levels (i.e., producing low of anti-HBs antibodies) do not show a CD4-positive T lymphocyte response to HBsAg.

The S1b peptide does appear to be a major epitope recognized by CD4-positive T lymphocytes; hence, helper/inducer T cell reactivity to S1b has been detected in 80% "responder" vaccinees, in 70% of subjects recovered from acute HBV infection and even in 40% of patients with chronic HBV infection. These latter results suggest that CD4-positive T lymphocytes reactive to S1b do not apparently react to the entire HBsAG molecule *in vitro* either: a) because S1b binds more efficiently than HBsAg to the surface of the antigen presenting cells of patients with chronic HBV infection; or b) because peptide Sib does not require antigen processing and binds directly to the HLA class II molecules of their antigen-presenting cells; or c) because HBsAg contains additional epitopes activating TS lymphocytes. Surprisingly, T cells reactive to the "T-cell R" peptide can not be detected in any subjects (including those with T cells reactive to Sib), suggesting interference of residues 17, 18, 34, 35, 36 with T cell reactivity and/or activation of T_s cells recognizing some of these residues.

In previous papers, others and myself have shown that CD8-positive T lymphocytes from patients with chronic HBV infection can specifically suppress the *in vitro* response to HBsAg of CD4-positive T cells of subjects recovered from acute hepatitis B [1, 3]. Thus I have performed two series of experiments to evaluate antigen-specific T_s cell activity in patients with chronic HBV infection and in "non responder" vaccinees.

First, using HBsAg as antigen, all chronic HBV carriers but only 20% "non responder" vaccinees have shown circulating CD8-positive T lymphocytes capable of specifically suppressing the response to the entire molecule of CD4-positive T cells of subjects recovered from acute hepatitis B [23]. Second, employing both S1b and "T-cell R" peptides in the same cultures, 70% of patients with chronic HBV infection and 20% "non responder" vaccinees have been found to have CD8-positive T cells capable of suppressing *in vitro* reactivity of CD4-positive T lymphocytes to the S1b peptide, suggesting that residues 17-18 and/or 34-36 can indeed activate CD8-positive T_s lymphocytes [23].

Taken together, the above results, although preliminary, strongly suggest that HBsAg contains both "helper" and "suppressor" epitopes: residues 19-33 (most likely 24-28) appear to represent an immunodominant "helper" determinant, whereas residues 17-18 and/or 34-36 seem to constitute a major "suppressor" epitope.

Conclusions

The identification of immunodominant helper and suppressor epitopes within HBsAg could open the way to the development of a peptide vaccine containing only the former and thus capable of: 1) promoting more sustained anti-HBs production in the "responder" population; and 2) increasing the number of "responders".

This latter goal however can be reached only to a limited extent, as the results obtained in the experiments summarized above clearly show that non responsiveness to the HBV vaccine does not depend, in the vast majority of cases, on T_s cells [23] and may rather be due to a defective antigen presentation or to "holes" in the T_H cell repertoire.

The enhancement of anti-HBs antibody responses in the "responder" population through the use of peptide vaccines is a fascinating possibility, but it appears difficult to achieve. Peptide vaccines offer limited number of B cell epitopes and thus need to stimulate powerful helper T cell responses. Hence the peptides need to be selected carefully to allow a range of different class II molecules to present them effectively. It has been reported that single peptides derived from viral antigens can stimulate helper/inducer T lymphocytes in the context of several MHC class II molecules [24, 25] and some data indicate that the CD4-positive T cell response to the S1 epitope of HBsAg may indeed be restricted by different MHC class II molecules in different individuals [21].

Only *in vivo* experiments with synthetic peptides such as S1 or S1b will clarify however whether the existing and highly effective HBsAg vaccines can be replaced by even more effective peptide vaccines.

Acknowledgements

The Author thanks Professor Adrian Eddleston (Liver Unit, King's College Hospital, London) for encouragement and helpful advice.

Review submitted on invitation by the Editorial Board of the *Annali*. Accepted for publication: June 15, 1990.

REFERENCES

1. VENTO, S., RONDANELLI, E.G., RANIERI, S., O'BRIEN, C.J., WILLIAMS, R. & EDDLESTON, A.L.W.F. 1987. Prospective study of cellular immunity to hepatitis B virus antigens from the early incubation phase of acute hepatitis B. *Lancet* **2**: 119-122.

2. SZMUNESS, W., STEVENS, C.E., ZANG, E.A., HARLEY, E.J. & KELLNER, A. 1981. A controlled clinical trial on the efficacy of the hepatitis B vaccine (Hepatavax): a final report. *Hepatology* **5**: 377-394.

3. VENTO, S., HEGARTY, J.E., ALBERTI, A., O'BRIEN, C.J., ALEXANDER, G.J.M., EDDLESTON, A.L.W.F. & WILLIAMS, R. 1985. T-lymphocyte sensitization to hepatitis B core Ag and T-cell-mediated unresponsiveness to hepatitis Bs Ag in hepatitis B virus-related chronic liver disease. *Hepatology* **5**: 192-197.

4. EDDLESTON, A.L.W.F. & WILLIAMS, R. 1974. Inadequate antibody response to HBAg or suppressor T-cell defect in the development of active chronic hepatitis. *Lancet* **2**: 1543-1545.

5. ROBERTS, I.M., BERNARD, C.C., VYAS, G.N. & MACKAY, I.R. 1975. T-cell dependence of immune response to hepatitis B surface antigen in mice. *Nature* **254**: 606-608.

6. CHANG, T.W., CELIS, E., MILLER, R.W., ZURAWSKY, V.R. Jr. & KUNG, P.C. 1984. *In vitro* response to HBsAg of peripheral blood lymphocytes from vaccine recipients of hepatitis B vaccine. *Hepatology* **4**: 824-829.

7. LE BOVIER, G.L. 1971. The heterogeneity of Australia antigen. *J. Infect. Dis.* **123**: 671-677.

8. BANCROFT, W.H., MUNDON, F.K. & RUSSELL, P.K. 1972. Detection of additional antigenic determinants of hepatitis B antigen. *J. Immunol.* **109**: 842-847.

9. IWARSON, S., TABOR, E., THOMAS, H.C., GOODALL, A., WATERS, J., SNOY, P., SHIH, W.K. & GERETY, R.J. 1985. Neutralization of hepatitis B virus infectivity by a murine monoclonal antibody: an experimental study in the chimpanzee. *J. Med. Virol.* **16**: 89-97.

10. SZMUNESS, W., STEVENS, C.D. & HARLEY, E.J. 1982. Hepatitis B vaccine in medical staff of haemodialysis units: efficacy and subtype cross-protection. *N. Engl. J. Med.* **307**: 1481-1483.

11. UNANUE, E.R. 1984. The regulation of lymphocyte functions by the macrophage. *Immunol. Rev.* **40**: 227-241.

12. MILICH, D.R., LEROUX-ROELS, G.G., LOUIE, R.E. & CHISARI, F.V. 1984. Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg). IV. Distinct H-2-linked Ir genes control antibody responses to different HBsAg determinants on the same molecule and map to the I-A and I-C subregions. *J. Exp. Med.* **159**: 41-51.

13. MILICH, D.R. & CHISARI, F.V. 1984. Immunogenetics and cellular correlates of the immune response to hepatitis B surface antigen determinants. In: *Advances in hepatitis research*. F.V. Chisari (Ed). Mason Publishing, New York. pp. 91-109.

14. CELIS, E., KUNG, P.C. & CHANG, T.W. 1984. Hepatitis B virus-reactive human T lymphocyte clones: antigen specificity and helper function for antibody synthesis. *J. Immunol.* **132**: 1511-1519.

15. DE LISI, C. & BERZOFSKY, J.A. 1985. T-cell antigenic sites tend to be amphipathic structures. *Proc. Natl. Acad. Sci. USA* **82**: 7048-7054.

16. MARGALIT, H., SPOUGE, J.L., CORNETTE, J.L., CEASE, K.B., DE LISI, C. & BERZOFSKY, J.A. 1987. Prediction of immunodominant helper T cell antigenic sites from the primary sequence. *J. Immunol.* **138**: 2213-2222.

17. ROTHBARD, J.B. 1986. Peptides and the cellular immune response. *Ann. Inst. Pasteur Virol.* **137E**: 518-525.

18. TOWNSEND, A., ROTHBARD, J., GOTCH, F.M., BAHADUR, G., WRAITH, D. & MCMICHAEL, A.J. 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* **44**: 959-968.

19. CHARNAY, P., POURCEL, C., LOUISE, A., FRITSCH, A. & TIOLLAIS, P. 1979. Cloning in *Escherichia coli* and physical structure of hepatitis B virion DNA. *Proc. Natl. Acad. Sci. USA* **76**: 2222-2227.

20. ONO, Y., ONDA, H., SASADA, R., IGARASHI, K., SUGINO, Y. & NISHIOKA, K. 1983. The complete nucleotide sequence of the cloned hepatitis B virus DNA; subtypes adr and adw. *Nucleic Acids Res.* **11**: 1747-1755.

21. CELIS, E., OU, D. & OTVOS, L. Jr. 1988. Recognition of hepatitis B surface antigen by human T lymphocytes. Proliferative and cytotoxic responses to a major antigenic determinant defined by synthetic peptides. *J. Immunol.* **140**: 1808-1815.

22. VENTO, S., HEGARTY, J.E., BOTTAZZO, G.F., MACCHIA, E., WILLIAMS, R. & EDDLESTON, A.L.W.F. 1984. Antigen-specific suppressor cell function in autoimmune chronic active hepatitis. *Lancet* **1**: 1200-1204.

23. VENTO, S., GAROFANO, T., CELIS, E., RAMAGE, B. & EDDLESTON, A.L.W.F. 1989. Identificazione di epitopi riconosciuti da linfociti T sull'antigene di superficie del virus epatitico B (HBV). In: *Atti XVII Convegno Nazionale GCI*. Cortona, 19-21 giugno. p. 43.

24. LAMB, J.R., MCMICHAEL, A.J. & ROTHBARD, J.B. 1987. T-cell recognition of influenza viral antigens. *Hum. Immunol.* **19**: 79-86.

25. HEBER-KATZ, E. & DIETZSCHOLD, B. 1986. Immune response to synthetic *Herpes simplex* virus peptides: the feasibility of a synthetic vaccine. *Curr. Top. Microbiol. Immunol.* **130**: 51-64.