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Autopresentation of Hepatitis B Virus Envelope Antigens by T Cells

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Processing and presentation by T cells appear to be limited to antigens that can directly interact with the T-cell surface, thereby overcoming the T-cell inefficiency in antigen capture and internalization. Our study provides evidence that the hepatitis B virus (HBV) envelope proteins can also be efficiently processed and presented by $CD4^+$ and $CD8^+$ T cells to other T cells in a human leukocyte antigen class II-restricted fashion. This phenomenon suggests a receptor-mediated interaction between T cells and the HBV envelope and defines a system that can, we hope, be exploited for the identification of the receptor binding site within the HBV envelope and for the characterization of the putative cellular HBV receptor.

The hepatitis B virus (HBV) is a hepatotropic virus which can also infect extrahepatic cells, such as lymphocytes (for a review, see reference 7). The mechanism of viral entry into the target cells is not known, although the interaction between amino acid sequences within the pre-S1 antigen of the HBV envelope and a still-unidentified cellular receptor on liver cells seems to be required for hepatocyte infection (12–14).

We reasoned that if a similar receptor-mediated mechanism is implicated in the interaction of the virus with T lymphocytes, then noninfectious HBV envelope particles, which are present in the blood at high concentrations during specific stages of HBV infection, should also be internalized by T cells.

It is believed that T cells can process exogenous antigens only when the antigen can interact with specific surface molecules, thereby gaining access to the intracellular endosomal processing compartment. This has recently been reported for the human immunodeficiency virus gp120 (10, 17), which can be presented by $CD4^+$ T cells to human leukocyte antigen (HLA) class II-restricted, gp120-specific T-cell clones (the CD4 molecule being its natural receptor [10]), and for the mouse κ light chain, which can be presented by $CD4^+$ and $CD8^+$ T cells to HLA class II-restricted, mouse κ light chain-specific T-cell clones, provided that the κ light chain is specifically targeted to T-cell surface molecules (10).

If such a specific interaction also takes place between T-cell surface structures and HBV envelope particles, T cells prepulsed with exogenous HBV envelope antigen should be able to internalize, process, and present it to HBV envelope-specific T cells in an HLA-restricted fashion without the need of "professional" antigen-presenting cells (APC), such as macrophages, dendritic cells, and B cells. This phenomenon could have important immunopathological consequences during HBV infection and could be exploited in vitro to define the features of the T-cell–virus interaction and to investigate the nature of the putative HBV receptor.

A preliminary indication that T cells can likely process and present native HBV envelope antigens was provided by HLA restriction experiments with HLA-matched or HLA-mismatched APC, designed to define the HLA molecule involved in antigen recognition by $CD4^+$ HLA class II-restricted pre-S1-specific T-cell clones previously produced in our laboratory (2).

The HBV envelope is composed of three different polypeptides with a common carboxy terminus encoded by a long open reading frame containing three in-phase translation start codons. The shortest polypeptide (S/p25), designated "major" because of its relative abundance in the viral particles, consists of 226 amino acids and expresses S reactivity. The middle envelope polypeptide (S+pre-S2) translated from the second in-phase initiation codon contains the entire amino acid sequence of the major envelope polypeptide with an additional 55 N-terminal residues expressing pre-S2 reactivity. The large envelope polypeptide (S+pre-S2+pre-S1) consists of the amino acid sequence of the middle envelope protein plus an additional 108 to 119 N-terminal amino acids (depending on subtype) containing the pre-S1 antigen (5).

When allogeneic peripheral blood lymphomononuclear cells (PBMC) as APC and large envelope proteins were incubated with $CD4^+$ T-cell clones for the entire period of culture, T-cell activation occurred irrespective of the HLA haplotype of the APC. In contrast, T-cell activation was selectively induced by HLA DQ1-positive APC when these cells were preincubated with HBV envelope antigens and then washed before coculture with the responder T-cell clones (data not shown). These results suggested that a direct interaction between T cells and HBV envelope antigens (without the need of conventional APC) could be sufficient to induce T-cell proliferation.

In order to investigate directly whether T cells can actually present HBV envelope antigens to other HBV envelope-specific T cells, we used $CD4^+$ HLA class II-restricted S- and pre-S1-specific T-cell clones both as APC and as responder cells in a conventional antigen-stimulated T-cell proliferation assay. $CD4^+$ T-cell clones as APC were preincubated with recombinant S+pre-S2+pre-S1 (rS+pre-S2+pre-S1) (6) and then washed and irradiated before coculture with effector T cells derived from the same clones. As shown in Fig. 1A, T cells were activated not only by antigen presented by Epstein-Barr virus-transformed B cells (EBV-B cells) but also by antigen presented by antigen-prepulsed T cells. The T-cell antigen-presenting function was expressed irrespective of the antigen specificity of the

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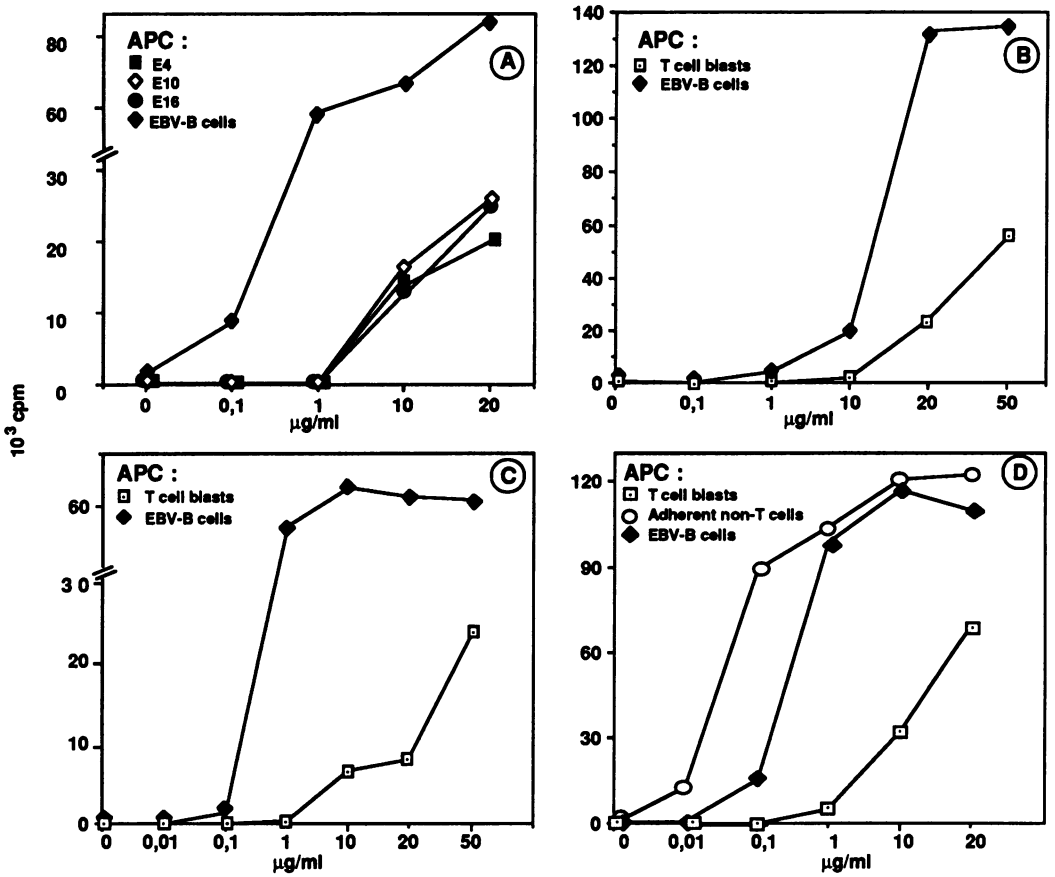

FIG. 1. Antigen presentation by autologous T cells, adherent non-T cells, and EBV-B cells. Irradiated or mitomycin C-treated APC ($CD4^+$ HLA class II-restricted HBV envelope-specific T cells from clones E4, E10, and E16 [A]; PHA-induced T-cell blasts [B through D]; EBV-B cells [A through D]; and adherent non-T cells [D]) were incubated for 5 h with the indicated concentrations of recombinant, yeast-derived S+pre-S2+pre-S1 and then washed and cocultured in round-bottomed well plates (1×10^5 T cells per well or 2×10^4 EBV-B cells or adherent non-T cells per well) with responder $CD4^+$ HBV envelope-specific T-cell clones (5×10^4 per well). [^{3}H]thymidine incorporation was evaluated after 3 days of culture.

presenting T cells, since phytohemagglutinin (PHA)-stimulated T-cell blasts were equally effective (Fig. 1B through D).

To avoid the possibility that contaminative non-T cells still present in culture after the previous T-cell stimulation were responsible for antigen presentation, T cells were always restimulated with PHA in the presence of allogeneic irradiated PBMC which were totally HLA mismatched with the responder T-cell clones.

The same stimulatory effect was observed when antigen-presenting T cells were incubated with antigen preparations enriched either in HBV DNA-defective 22-nm envelope particles (human [h]S+pre-S2+pre-S1) (3) or in HBV virions (Dane particles, kindly provided by M. A. Petit) (13), both containing pre-S1, and purified from sera of chronic HBV carriers (Fig. 2A and 3). These results indicated that T-cell activation was not an artifact related to the specific nature of the recombinant HBV envelope antigen preparation used in the initial experiments.

In addition, $CD4^+$ S-specific T-cell clones could not be stimulated by major envelope proteins of three different origins expressing only S reactivity (plasma purified [11] or produced in yeast [4] or in Chinese hamster ovary [CHO] cells) when T cells were used as APC, even though the same antigen preparations were clearly stimulatory when presented to the same T-cell clones by EBV-B cells (Fig. 3). This finding shows that these antigen preparations cannot be appropriately presented by T cells even though they are immunogenic at the T-cell level, as shown by EBV-B-cell presentation. In contrast, T-cell presentation of large envelope proteins, which also contain pre-S antigens, was highly efficient (Fig. 3), suggesting that critical residues needed for antigen interaction with T cells are likely contained within the pre-S region of the HBV envelope. In addition, the capacity of T cells to present large but not major envelope proteins to S-specific T-cell clones further confirms that antigen presentation by T cells is not an artifact related to the presence of contaminative autologous non-T cells.

That T-cell activation by HBV large envelope proteins requires intracellular processing was demonstrated by blocking antigen presentation by glutaraldehyde fixation or chloroquine treatment of the antigen-presenting T cells. As shown in Fig. 4, antigen presentation was inhibited by fixation of the APC before but not after incubation with antigen. In contrast, no inhibition of the presentation of a specific peptide was observed. In agreement with these results, chloroquine treatment (200 µM) of the antigen-presenting T cells showed an inhibitory effect on the presentation of the whole HBV envelope proteins but not of a short synthetic envelope peptide (data not shown).

Antigen presentation by T cells is an HLA-restricted

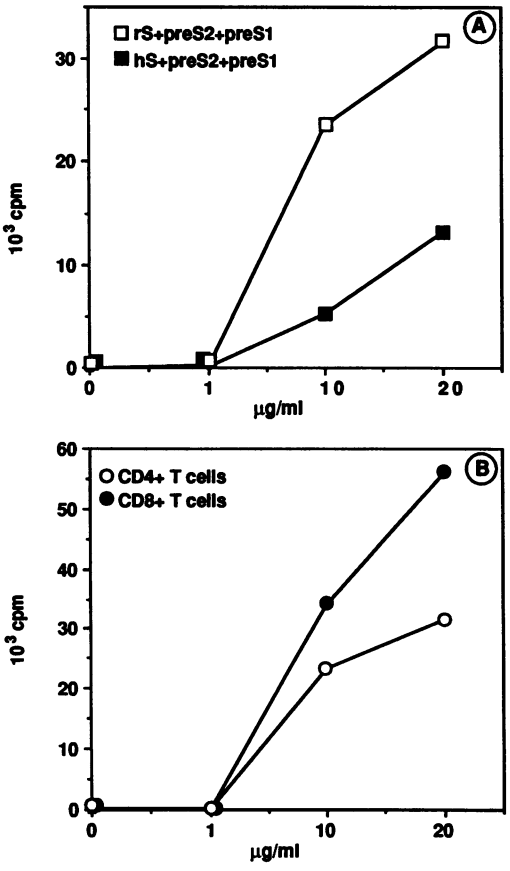

FIG. 2. (A) Presentation of rS+pre-S2+pre-S1 and hS+pre-S2+pre-S1 by PHA-induced T-cell blasts to a responder $CD4^+$ pre-S1-specific T-cell clone. Experimental conditions were as described in the legend to Fig. 1. (B) Presentation of rS+pre-S2+pre-S1 by $CD4^+$ and $CD8^+$ T cells to a representative $CD4^+$ pre-S1-specific T-cell clone. Nonadherent, E-rosetting T cells were separated into $CD8^+$ and $CD4^+$ T cells by panning with anti-CD4 or anti-CD8 monoclonal antibodies (18). Positively selected T-cell subsets (more than 95% pure) were stimulated with PHA in the presence of irradiated (5,000 rads) allogeneic PBMC (HLA class II mismatched with the HBV envelope-specific T-cell clones). After at least two complete rounds of PHA stimulation, $CD8^+$ T cells (4% of contaminative $CD4^+$ T cells as determined by fluorescence-activated cell sorter [FACS] analysis) and $CD4^+$ T cells (1% of contaminative $CD8^+$ T cells) were used as APC (10^5 per well) for HBV envelope T-cell clones (5×10^4 per well). Results obtained with representative $CD4^+$ T-cell clones are shown. For culture conditions, see the legend to Fig. 1. The possibility that presentation by the $CD8^+$ T-cell line was due to contaminative $CD4^+$ T cells was further ruled out by the absence of T-cell activation when a number of $CD4^+$ T-cell blasts equivalent to that present among the $CD8^+$ T-cell population (less than 5%, on the basis of FACS analysis; 5×10^4 per well) was used as APC (data not shown).

phenomenon, since only allogeneic, activated T cells expressing the relevant HLA restriction molecule needed for antigen recognition by T cells can function as APC. This is shown by the observation that DQ1-positive but not DQ1-negative T-cell blasts can present the pre-S1 antigen to DQ1-restricted, pre-S1-specific T-cell clones and lines (Fig. 5).

Interestingly, both $CD4^+$ and $CD8^+$ T-cell blasts were able to present HBV envelope antigens to T-cell clones with equal efficiency (Fig. 2B). In contrast, only $CD4^+$ T cells can process and present the human immunodeficiency virus gp120 antigen, since the CD4 molecule represents the specific cellular receptor for gp120. This suggests that a receptor for HBV envelope antigens could be expressed on both $CD4^+$ and $CD8^+$ T cells.

The antigen-presenting capacity of the T cells was less efficient than the capacities expressed by EBV-B cells and adherent non-T cells, since antigen concentrations required for T-cell activation by antigen-presenting T cells were usually approximately 100 times higher than those required by B cells and macrophages (Fig. 1).

From all the experiments described above, we conclude that T cells can process and present the HBV envelope antigens.

This phenomenon could have several biological implications. Since all activated T cells are potentially able to express this processing function irrespective of their antigen specificities and phenotypes, one could hypothesize that this mechanism is useful in amplifying the T-cell response to HBV envelope antigens, especially in stages of HBV infection when envelope particles are abundantly present in the blood.

On the other hand, the processing capacity could make T

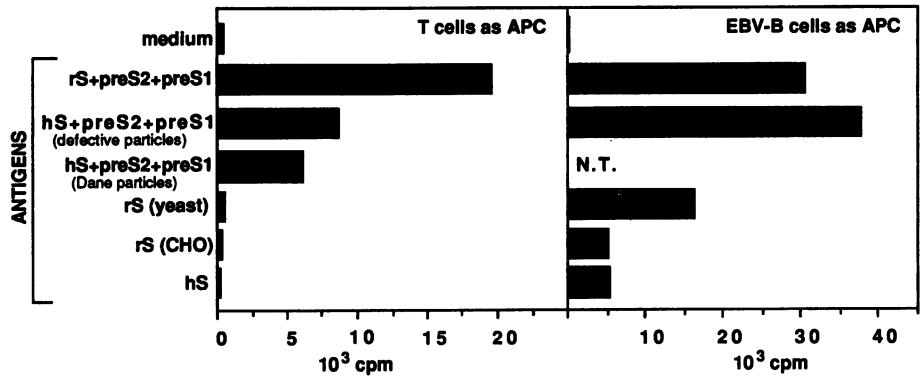

FIG. 3. Presentation of different HBV envelope antigen preparations (recombinant and human plasma-purified S+pre-S2+pre-S1 and recombinant yeast- or CHO-derived and human plasma-purified S antigen) by a $CD4^+$ T-cell clone (left) and by EBV-B cells (right) to a $CD4^+$ S-specific T-cell clone. Antigens were used at high concentrations (20 µg/ml). N.T., not tested.

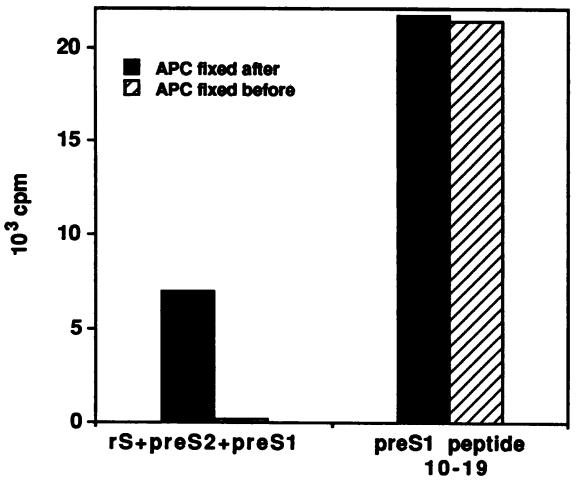

FIG. 4. T-cell presentation of HBV large envelope proteins is abrogated by glutaraldehyde fixation. PHA-induced T-cell blasts were fixed by incubation in 0.05% glutaraldehyde (Serva, Heidelberg, Germany) for 30 s, and the reaction was stopped by the addition of 0.2 M glycine. T cells were fixed either before or after incubation (5 h) with rS+pre-S2+pre-S1 or with the pre-S1 peptide 10-19, specifically recognized by the responder $CD4^+$ pre-S1-specific T-cell clone E10. After being washed, 10^5 fixed APC were cultured with 5×10^4 T cells from the E10 clone.

cells potential targets for the cytolytic activity of $CD4^+$ HLA class II-restricted HBV envelope-specific cytotoxic T cells. Whether this phenomenon can actually lead to a down-regulation of the immune response by a mechanism similar to that described for gp120-specific T cells in human immunodeficiency virus infection (17) remains to be determined, since the relevance in vivo of the HLA class II-restricted cytotoxic activity is still a debated issue (1, 9, 15, 16). With regard to this, the evidence that highly viremic patients with chronic HBV infection (HBVe antigen positive) are not strongly immunosuppressed, despite the high circulating levels of HBV envelope particles (which could target T cells for destruction by envelope-specific cytotoxic T cells), apparently supports the view that this HLA class II-restricted cytolytic activity is not actually expressed in vivo, even though it is frequently observed in vitro after expansion in the presence of exogenous interleukins.

Alternatively, the lack of strong immunosuppression in these patients could also be explained by the low efficiency of the T-cell antigen-presenting function, which in our system is at least 2 orders of magnitude lower than that of conventional APC.

This low efficiency may be related to the density of the putative surface receptor on T cells, the nature of the receptor itself, and the affinity of the receptor-ligand interaction. For instance, the efficiency of antigen presentation by B cells varies in relation to the surface molecule which serves as a receptor for antigen, being maximal when en-

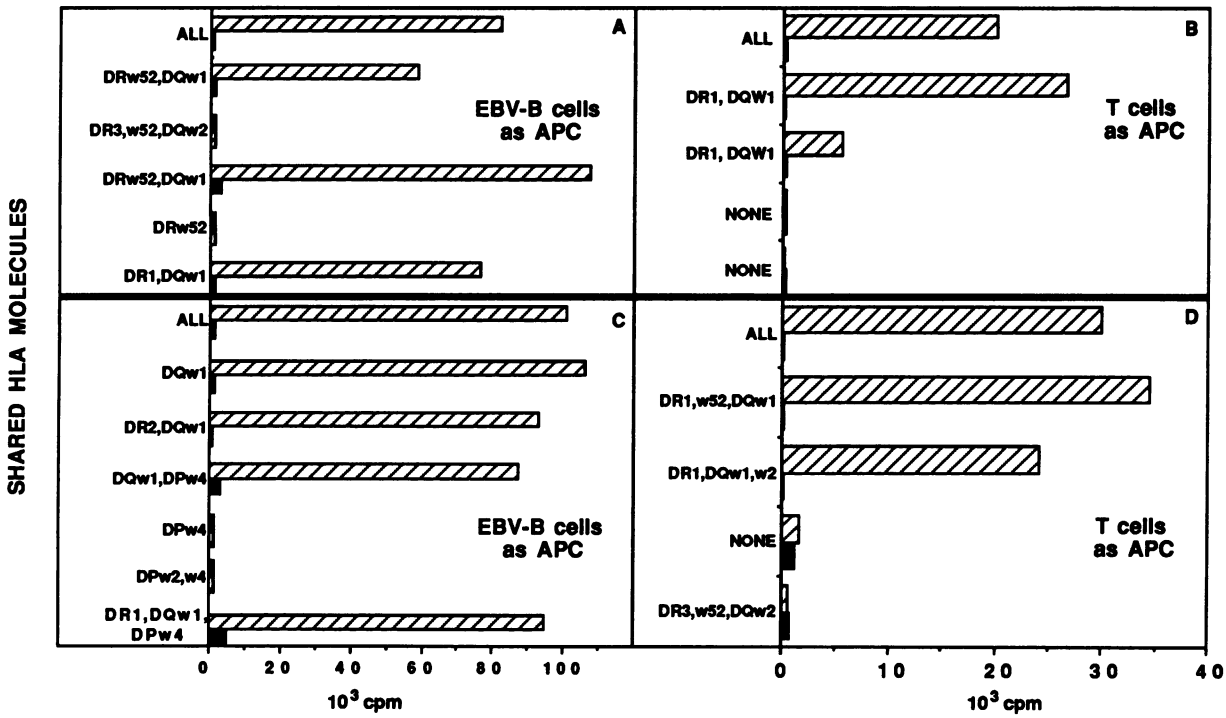

FIG. 5. HLA restriction of antigen presentation by T cells. Autologous and allogeneic mitomycin C-treated EBV-B cells (A and C) or irradiated (3,000 rads) PHA-stimulated T-cell lines (B and D) were cultured overnight with rS+pre-S2+pre-S1 or with medium alone. After incubation, the APC were washed and added to 5×10^4 effector cells from a DQ1-restricted, pre-S1-specific polyclonal T-cell line (A and B) and from a DQ1-restricted pre-S1-specific T-cell clone (C and D). All polyclonal T-cell lines were kept in culture for at least two rounds of PHA restimulation in the presence of allogeneic irradiated PBMC previously shown to be unable to present antigen to the envelope-specific T-cell lines and clones before being used in the restriction experiments. [^{3}H]thymidine incorporation was measured after 3 days of culture. The pre-S1-specific T-cell line was derived from a subject which was HLA DR1,3,w52, DQw1,w2 positive (DP typing was not available). The pre-S1-specific T-cell clone was produced from a subject which was HLA DR1,2, DQw1, DPw2,w4 positive. The HLA molecules shared by the allogeneic APC with the T-cell donors are indicated. ▨, rS+pre-S2+pre-S1; ■, medium.

docytosis is mediated by surface immunoglobulins but lower when it is mediated by HLA class II and the transferrin receptor and minimal when it is mediated by HLA class I (8).

Finally, the use of different HBV envelope antigen preparations containing different proportions of the various envelope region-encoded polypeptides to activate T cells in this system should allow the identification of the amino acid sequences relevant to HBV interaction with T cells. Moreover, this system could also be exploited to elucidate the nature of the putative cellular receptor trying to block the T-cell–antigen interaction with monoclonal antibodies recognizing specific molecules expressed on the T-cell surface.

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REFERENCES

1. **Del Prete, G. F., M. De Carli, M. Ricci, and S. Romagnani.** 1991. Helper activity for immunoglobulin synthesis of T helper type 1 (Th1) and Th2 human T cell clones: the help of Th1 clones is limited by their cytolytic capacity. J. Exp. Med. **174:**809–813.
2. **Ferrari, C., A. Penna, A. Bertoletti, A. Cavalli, A. Valli, C. Schianchi, and F. Fiaccadori.** 1989. The preS1 antigen of hepatitis B virus is highly immunogenic at the T cell level in man. J. Clin. Invest. **84:**1314–1319.
3. **Ferrari, C., A. Penna, P. Sansoni, T. Giuberti, T. M. Neri, F. V. Chisari, and F. Fiaccadori.** 1986. Selective sensitization of peripheral blood T lymphocytes to hepatitis B core antigen in patients with chronic active hepatitis type B. Clin. Exp. Immunol. **67:**497–506.
4. **Hauser, P., P. Voet, E. Simoen, H. C. Thomas, J. Petre, M. De Wilde, and J. Stephenne.** 1987. Immunological properties of recombinant HBsAg produced in yeast. Postgrad. Med. J. **63:**(Suppl. 2):83–94.
5. **Heermann, K. H., U. Goldmann, W. Schwartz, T. Seyffarth, H. Baumgarten, and W. H. Gerlich.** 1984. Large surface proteins of hepatitis B virus containing the pre-*s* sequence. J. Virol. **52:**396–402.
6. **Kniskern, P. J., A. Agopian, P. Burke, N. Dunn, E. A. Emini, W. J. Miller, S. Yamazaki, and R. W. Ellis.** 1988. A candidate vaccine for hepatitis B containing the complete viral surface protein. Hepatology **8:**82–87.
7. **Korba, B. E., F. Wells, B. C. Tennant, G. H. Yoakum, R. H. Purcell, and J. L. Gerin.** 1986. Hepadnavirus infection of peripheral blood lymphocytes in vivo: woodchuck and chimpanzee models of viral hepatitis. J. Virol. **58:**1–8.
8. **Lanzavecchia, A.** 1987. Antigen uptake and accumulation in antigen-specific B cells. Immunol. Rev. **99:**39–51.
9. **Lanzavecchia, A.** 1989. Is suppression a function of class II-restricted cytotoxic T cells? Immunol. Today **10:**157–159.
10. **Lanzavecchia, A., E. Roosnek, T. Gregory, P. Berman, and S. Abrignani.** 1988. T cells can present antigens such as HIV gp120 targeted to their own surface molecules. Nature (London) **334:**530–532.
11. **Milich, D. R., D. L. Peterson, G. G. Leroux-Roels, R. A. Lerner, and F. V. Chisari.** 1985. Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg). VI. T cell fine specificity. J. Immunol. **134:**4203–4211.
12. **Neurath, A. R., S. B. H. Kent, N. Strick, and K. Parker.** 1986. Identification and chemical synthesis of a host cell receptor binding site on hepatitis B virus. Cell **46:**429–436.
13. **Petit, M. A., F. Capel, and J. Pillot.** 1985. Demonstration of a firm association between hepatitis B surface antigen proteins bearing polymerized human albumin binding sites and core-specific determinants in serum hepatitis B viral particles. Mol. Immunol. **22:**1279–1287.
14. **Pontisso, P., M.-A. Petit, M. J. Bankowski, and M. E. Peeples.** 1989. Human liver plasma membranes contain receptors for the hepatitis B virus pre-S1 region and, via polymerized human serum albumin, for the pre-S2 region. J. Virol. **63:**1981–1988.
15. **Romagnani, S.** 1991. Human Th1 and Th2 subsets: doubt no more. Immunol. Today **12:**256–257.
16. **Scott, P., and S. H. E. Kaufmann.** 1991. The role of T-cell subsets and cytokines in the regulation of infection. Immunol. Today **12:**346–348.
17. **Siliciano, R. F., T. Lawton, C. Knall, R. W. Karr, P. Berman, T. Gregory, and E. L. Reinherz.** 1988. Analysis of host-virus interactions in AIDS with human T cell clones specific for HIV gp120: effect of HIV genomic heterogeneity and a mechanism for CD4+ cell depletion. Cell **54:**561–575.
18. **Wysoki, L. J., and U. L. Sato.** 1978. Panning for lymphocytes. A method for cell separation. Proc. Natl. Acad. Sci. USA **75:** 2844–2847.