sists of an 80-kD cell surface EGF binding portion with 30 kD of oligosaccharide (present in ERRP) and a 65-kD cytoplasmic tyrosine kinase and transmembrane containing portion (missing in ERRP). The amino-terminal sequence homologies indicate that the orientation of the EGF receptor is NH_2–EGF receptor–transmembrane–tyrosine kinase (erb B)–COOH.

ERRP does not appear to be derived from degradation of intact cell membrane inserted 170-kD EGF receptor. Only a fraction of the EGF receptor–kinase may mature to the membrane bound protein, and ERRP may be generated from proteolytic processing within the endoplasmic reticulum and Golgi. If this is correct, a domain containing tyrosine kinase (erb B) would be produced and either degraded or transported as a separate protein. By analogy with the insulin receptor whose tyrosine kinase activity is increased by partial proteolysis (*31*), separation of an erb B homolog from the EGF receptor portion might result in activation of the former. Alternatively, the EGF receptor–kinase and ERRP could be produced by way of separate genes or alternative splicing pathways. Using short 15-minute pulses of [^{35}S]methionine and a monoclonal anti-EGF receptor antibody, Cooper *et al.* (*32*) detected a slightly smaller precursor that matured to the 170-kD EGF receptor protein. Analysis of figure 7 from that study reveals a separate protein of ~ 100 kD which was synthesized in about the same amounts as EGF receptor protein but was not chased into mature EGF receptor by excess unlabeled methionine, rather it disappeared from the cell, consistent with it being secreted. Recent unpublished data indicates that A431 cells contain at least two EGF receptor–erb B messenger RNA (mRNA) species, a finding in agreement with that reported for chick cells which contain two mRNA's with erb B sequences (*33*). Taken together, these results suggest that ERRP is made separately from the EGF receptor. Synthesis may be from a distinct mRNA generated via alternative splicing.

It remains possible that EGF receptor degradation (*34*), as well as separate synthesis, contributes to total ERRP detected in medium, and additional studies are required to determine whether production of ERRP occurs in normal cells or is restricted to tumor cells. In A431 cells, increased synthesis of both EGF receptors and ERRP may result from translocation of the region of chromosome 7 containing the EGF receptor (*35*) and erb B (*36*) genes and their amplification. The secreted ERRP may provide a readily measurable marker of excessive production of EGF receptor and erb B proteins in human epidermoid carcinomas.

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Antibodies to Hepatitis B Surface Antigen Potentiate the Response of Human T Lymphocyte Clones to the Same Antigen

Abstract. *Human T-helper lymphocyte clones specific for hepatitis B virus surface antigen (HBsAg) proliferate on stimulation with HBsAg in vitro. Antibodies specific for HBsAg, but no other antibodies, augment this proliferative response. In the presence of antibodies to HBsAg, the maximum response could be achieved at HBsAg concentrations that were 1 percent of those required in the absence of the antibodies. These findings suggest that antigen-specific antibodies exert regulatory controls on T cells that recognize the same antigens.*

T lymphocytes are important in the regulation of immune responses to most antigens (*1*). One characteristic of T cells is that they recognize antigens only in association with major histocompatibility gene complex–encoded molecules (Ia or DR) present on the surface of accessory cells (*2*) known as antigen-presenting cells. After T lymphocytes are triggered by the antigen, they proliferate and secrete factors that eventually will enhance or suppress the production of immunological effector molecules and cells (for example, antibodies and cytotoxic T lymphocytes).

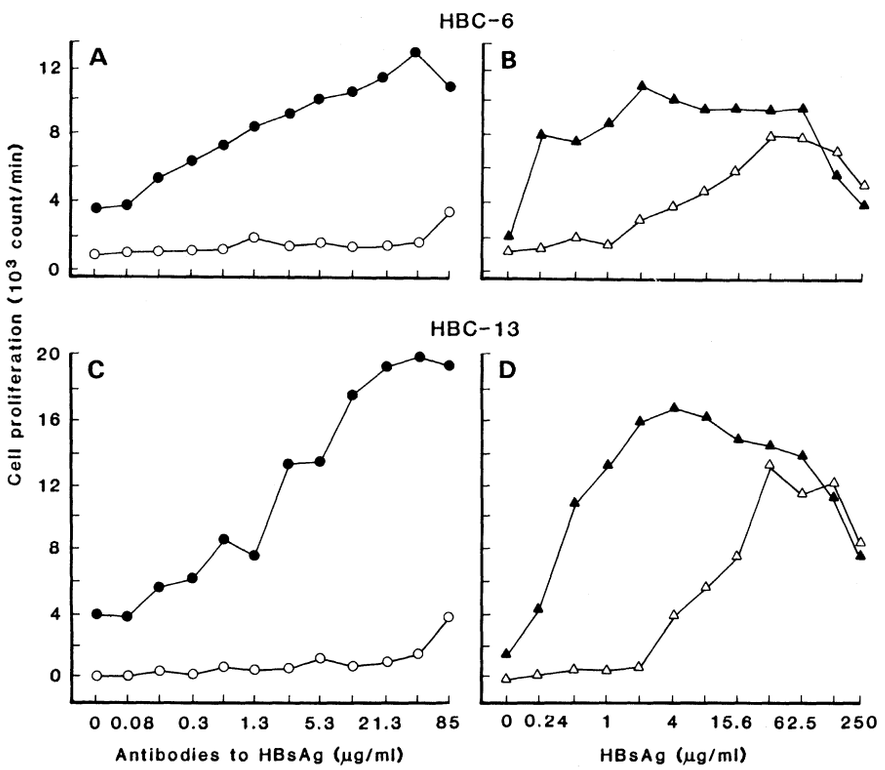
As with most infectious diseases, resistance to hepatitis B virus (HBV) is largely mediated by protective antibodies and immune effector cells (*3*). Individuals who recover from an acute HBV infection develop immunity to the virus by producing antibodies to the viral en-

velope molecule, hepatitis B surface antigen (HBsAg) (*3*). In fact, individuals with a high risk of HBV infection have been successfully immunized against HBV with an inactivated, purified preparation of HBsAg. Most of the vaccine recipients develop high titers of antibodies to HBsAg, and the incidence of hepatitis B is decreased markedly among vaccinated individuals (*4*).

We studied the response of lymphocytes obtained from hepatitis B vaccine recipients to HBsAg in vitro (*5*). We used antigen and T cell growth factor to develop HBsAg-specific T lymphocyte clones from the peripheral blood mononuclear cells of vaccine recipients. These clones proliferated specifically in response to HBsAg, and some of them proved to be helper T cells; that is, they promoted the production in vitro of antibodies to HBsAg by HBsAg-specific autologous B lymphocytes (*5*). These helper clones, when stimulated by antigen (*6*), secrete the immunoregulatory factors interferon-γ and B cell growth factors.

Because T lymphocytes do not bind antigens directly, it seems likely that the antibodies reactive with HBsAg and the HBsAg-specific lymphocytes do not recognize the same epitopes on HBsAg molecules. Since antibodies to HBsAg are present for a long time in the circulation of vaccine recipients and of previously HBV-infected but healthy individuals, we were interested in studying the effects that the antibodies may have on the reactivity of T lymphocytes to HBsAg under well-defined conditions in vitro. We analyzed two HBsAg-specific human T-helper lymphocyte clones (HBC-6 and HBC-13). Antibodies to HBsAg were obtained by affinity chromatography of plasma from individuals immunized with HBV vaccines; agarose beads conjugated with purified HBsAg were used for the affinity purification. The proliferative response was measured by the incorporation of [^{3}H]thymidine into the DNA of dividing T cells after 2 to 3 days of incubation with the antigen and antibody.

Antibodies to HBsAg dramatically increased the proliferative response of the T-helper cell clones, HBC-6 and HBC-13, to HBsAg (5 μg/ml) in a dose-related manner (Fig. 1, A and C). Antibodies alone produced a small but significant increase of the proliferative response of



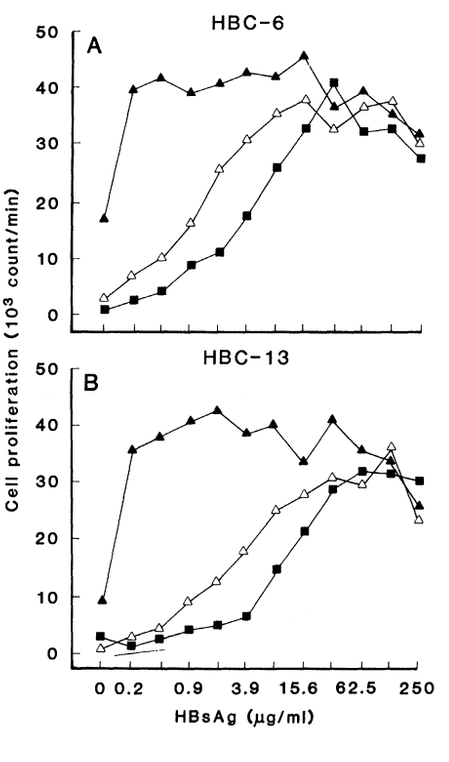


Fig. 1 (left). Effects of antibodies to HBsAg in antigen-induced proliferation of HBsAg-specific T cell clones. Antibodies to HBsAg were obtained by affinity chromatography of plasmas from recipients of hepatitis B vaccine. Cross-linked agarose (Affi-Gel 10; Bio-Rad, Richmond, California) coupled with HBsAg was used for chromatography. A sensitive radioimmunoassay showed that the eluted antibodies were free from HBsAg (less than 0.1 ng of HBsAg per 200 μg of antibodies to HBsAg). HBsAg was obtained by affinity chromatography of plasmas from chronic carriers of hepatitis B. Affi-Gel 10 coupled with the mouse monoclonal antibody 5D3, which specifically reacts with HBsAg (*14*), was used for chromatography. Proliferation was measured by incubating 2×10^4 cloned T cells and 1×10^5 irradiated autologous peripheral blood mononuclear cells with different concentrations of HBsAg and antibodies to HBsAg in 96-well flat-bottom trays (Costar) in a final volume of 200 μl of RPMI 1640 tissue culture medium that contained 10 percent fetal calf serum, 25 m*M* Hepes, 5 m*M* L-glutamine, 5×10^{-5}*M* 2-mercaptoethanol. After a 54-hour incubation at 37°C in an environment of 5 percent CO_2 and 95 percent humid air, each culture was treated with 1 μCi of [^{3}H]thymidine for 18 hours under the same conditions. The amount of ^{3}H incorporated into DNA was determined by liquid scintillation spectrography after the cultures were harvested onto glass-fiber filters. The effects of different amounts of antibodies to HBsAg in the antigen-mediated proliferation was studied with clones HBC-6 (A) and HBC-13 (C) in the presence of HBsAg (5 μg/ml) (●) and in the absence of HBsAg (○). The proliferation of HBC-6 (B) and HBC-13 (D) induced by different concentrations of HBsAg was determined in the presence of antibodies to HBsAg (20 μg/ml) (▲) and in the absence of the antibodies (△). Each point represents the mean of duplicate determinations and the standard error of the mean for each point was less than 10 percent of the mean. Fig. 2 (right). Effect of antibodies of different antigenic specificities on HBsAg-induced proliferation of antigen-specific T cell clones. The procedures for the purification of HBsAg and antibodies to HBsAg and for determining the degree of proliferation were as described in Fig. 1. Antibodies to tetanus toxoid were purified from plasmas obtained from volunteers recently immunized with tetanus vaccine; Sepharose 4B (Pharmacia, Piscataway, New Jersey) coupled with tetanus toxoid (State Laboratory, Commonwealth of Massachusetts, Boston) was used for affinity chromatography. HBC-6 (A) and HBC-13 (B) cells were incubated with different concentrations of HBsAg in the presence of antibodies to HBsAg (20 μg/ml) (▲), in the presence of antibodies to tetanus toxoid (■), or in the absence of antibodies (△). Each point represents the mean of duplicate determinations and standard error of the mean for each point was less than 10 percent of the mean.

both clones. The proliferative response to varying concentrations of HBsAg was also measured in the presence of a constant amount of antibodies to HBsAg. In the presence of antibodies to HBsAg (20 μg/ml), the maximum response to HBsAg could be achieved at antigen concentrations that were 1 to 10 percent of the concentrations required in the absence of the antibodies (Fig. 1, B and D).

In a separate experiment, we examined the effects of irrelevant antibodies—purified human antibodies to tetanus toxoid—on the HBsAg-induced proliferation of the helper T cell clones. The two T cell clones were specific for HBsAg and did not respond to tetanus toxoid (*5*). Antibodies to tetanus toxoid slightly inhibited the proliferative response (Fig. 2). The results indicate that the effect of antibodies to HBsAg on the T cell response to HBsAg is antigen-specific.

The exact mechanism by which the antibodies to HBsAg increase the response of the T lymphocyte clones to HBsAg has not been elucidated. One explanation is related to the ability of antibodies to polymerize and aggregate the antigen with which they react. The number of antigenic determinants or epitopes per molecule (valence) is higher in the polymer form, and this antigenic valence is directly proportional to the apparent binding affinity of the surface receptors for the antigen (*7*). One of us (E.C.) showed earlier that the binding of polyvalent antigen to solid phase–bound receptors can be regulated by soluble antibodies either competitively or synergistically, depending on the relative antibody concentration (*8*).

However, HBsAg and the antigen-antibody complexes probably do not bind directly to the antigen receptor on the T lymphocyte surface, and antigen-presenting cells such as macrophages and monocytes appear to play an important role in the antigen stimulation. Antigens aggregated by antibodies may be more easily captured than soluble antigens by these phagocytic cells. Additionally, the immunoglobulins in the immune complexes may bring them to the antigen-presenting cells through binding to the surface Fc receptors (*9*). The involvement of Fc receptors on monocyte interaction with immunoglobulins has been suggested in the studies of human T cell proliferation induced by monoclonal antibody OKT3 (*10*).

Our results suggest that antibodies may sustain or amplify normal immune responses in vivo by exerting the augmenting effects on T-helper cells. This positive-feedback effect of antibodies would most likely occur in secondary immune responses when some amounts of the specific antibody are already present in the circulation.

Several groups (*11*) have observed that antibodies to HBsAg are generated in some individuals given exogenous HBV-specific immunoglobulins. It was postulated that the small amounts of HBsAg present in some immunoglobulin preparations induced this "passive-active immunization" (*11*). Our results indicate that this suggestion may be correct since low concentrations of HBsAg become immunogenic when complexed with antibody to HBsAg, and an efficient immune response to HBsAg was observed in vitro even at high concentrations of antibodies (Fig. 1, A and C).

The role of antibodies in regulating immune responses in vivo has been studied by several groups. Although some reported that antigen-specific antibodies administered separately, or together with the immunogens, potentiate antibody responses (*12*), others found opposite results (*13*). The cellular mechanism responsible for these regulatory effects of antibodies has not been clearly characterized. Our present in vitro studies with helper T cell clones provide an explanation for some of the in vivo findings. It would be interesting to study whether the activities of antigen-specific T suppressor cells are also regulated by antibodies recognizing the same antigen and whether the opposing effects of antibodies in vivo are due to the unequal augmentation of helper and suppressor T cells.

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Ancient Ice Islands in Salt Lakes of the Central Andes

Abstract. *Massive blocks of freshwater ice and frozen sediments protrude from shallow, saline lakes in the Andes of southwestern Bolivia and northeastern Chile. These ice islands range up to 1.5 kilometers long, stand up to 7 meters above the water surface, and may extend out tens of meters and more beneath the unfrozen lake sediments. The upper surfaces of the islands are covered with dry white sediments, mostly aragonite or calcite. The ice blocks may have formed by freezing of the fresh pore water of lake sediments during the "little ice age." The largest blocks are melting rapidly because of possibly recent increases in geothermal heat flux through the lake bottom and undercutting by warm saline lake water during the summer.*

Between the eastern and western ranges of the Central Andes lies a broad, high basin of internal drainage known as the altiplano. Lake Titicaca is at the northern end, and Salar de Uyuni, a 9000 km^2 salt flat, dominates the central and lowest (3650 m above sea level) portion. At the southern end, the altiplano rises in elevation and is broken into a large number of small isolated basins, most of which contain one or more salt lakes (*1–3*). In ten of the several dozen lakes examined, we have found massive blocks of freshwater ice and frozen sedi-