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4. The International Astronomical Union (IAU), as of this writing, has not officially recognized the existence of 1978 P1, although it is expected to do so at the General Assembly meeting in late 1985. Until then, the name "Charon" remains unofficial. In Greek mythology, Charon was the boatman who ferried souls to the realm of Hades across the river Styx; Pluto was, of course, the Roman aspect of the Greek god Hades.
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Enhanced Immunogenicity of the Pre-S Region of Hepatitis B Surface Antigen

Abstract. *The 55 codons upstream of the gene sequence encoding the hepatitis B surface antigen (HBsAg) are called the pre-S(2) region. It has been proposed that polypeptides of high molecular weight that contain the pre-S(2) region should be included in future hepatitis B virus (HBV) vaccines. The pre-S(2) region and the S gene product [25 kilodalton (kD)] together compose a polypeptide of high molecular weight (33 kD). As an initial attempt to determine the relevance of the 33-kD polypeptide to development of an HBV vaccine, the murine immune response to pre-S(2)–encoded determinants as compared to S-encoded determinants on the same polypeptide was examined. The results indicate (i) the pre-S(2) region is significantly more immunogenic than the S region of HBsAg, (ii) the 26 amino acid residues at the NH_2-terminus of the 33-kD polypeptide represent a dominant antibody binding site on the pre-S(2) region, (iii) the immune response to the pre-S(2) region is regulated by H-2–linked genes distinct from those that regulate the response to the S region, and (iv) immunization of an S region nonresponder strain with HBV envelope particles that contain both the pre-S(2) and S regions can circumvent nonresponsiveness to the S region.*

Immunologic markers of hepatitis B virus (HBV) include the surface antigen (HBsAg), the core antigen (HBcAg), and the core-derived HBeAg. Since HBsAg-specific antibodies are protective against HBV infection, virus-free envelopes present in the plasma of chronic carriers have served as a source of HBV vaccines. The HBsAg is composed of a major polypeptide, p25, and its glycosylated form, gp28 (*1*). The complete 226 amino acid sequence of the p25 polypeptide of HBsAg has been deduced from partial amino acid sequence data (*1*) and from the nucleotide sequence of the viral gene (S) that encodes this polypeptide (*2–4*). Additional polypeptides of higher molecular weight associated with HBsAg have been considered aggregates of p25 and gp28; however, p25 begins at the third possible translational initiation site of a larger open reading frame (ORF) and is preceded in phase by 163 or 174 codons (subtype-dependent) designated the pre-S region (*5*). An HBV-associated 33- to 36-kilodalton (kD) glycoprotein (gp33) has been identified (*6*), and it has been suggested that the sequence of gp33 starts at the second translational initiation signal of the ORF, which is 55 codons upstream from the third signal (*7*). It has been shown that gp33 consists of the p25 sequence and a sequence of 55 amino acid residues at the NH_2-terminus encoded in this pre-S(2) region (*8, 9*). In support of this, Neurath and co-workers synthesized a peptide encompassing the 26 amino acid residues at the NH_2-terminus of the pre-S(2) region, and antibodies to this peptide reacted with gp33 (*10*).

The fact that the pre-S region is conserved in all described HBV DNA sequences and conserved through evolution (*11*) suggests a functional role for this region. This interpretation is supported by the observation that gp33 appears to be preferentially expressed in viremic carriers as opposed to carriers with minimal or no infectious virions in the blood (*8, 12*). This suggests a correlation between viral replication and synthesis of the higher molecular weight polypeptides of HBsAg. Additionally, the receptor for polymerized human albumin, which has been suggested to mediate viral attachment, has been localized on gp33 (*13*). The implications of these recent findings relative to HBV vaccine development and to mechanisms of immune-mediated viral clearance prompted us to investigate the immune response to the S- and pre-S(2)–encoded gp33 polypeptide of HBsAg.

In previous studies of the murine humoral and cellular immune response to HBsAg, we demonstrated the influence of at least two H-2–linked immune response (Ir) genes and identified HBsAg high-responder ($H\text{-}2^d$ and $H\text{-}2^q$), and nonresponder ($H\text{-}2^f$ and $H\text{-}2^s$) haplotypes (*14–16*). The HBsAg used in those studies contained the p25 and gp28 polypeptides but lacked the higher molecular weight polypeptides coded for by the pre-S region, here designated as HBsAg/p25. In the present studies we compared the immune responses to pre-S(2)–encoded determinants and S-encoded determinants of HBsAg in terms of immunogenicity, specificity, H-2–linked regulation, and possible overlapping regulatory mechanisms (that is, whether or not the T-cell helper function generated in response to the pre-S region would influence the anti-S response). For this purpose we used HBsAg particles derived from Chinese hamster ovary (CHO) cells transfected with a plasmid containing the S gene and the pre-S region of HBV (*17*); these particles are designated HBsAg/p34. The HBsAg/p34 particles are composed of the S-encoded p25/gp28 polypeptides plus the pre-S(2)– and S-encoded gp34 polypeptide. The gp34 polypeptide corresponds to HBV gp33 (*17*).

Groups of mice from a panel of H-2 congenic strains were immunized intraperitoneally with 1.0 μg of HBsAg/p34 emulsified in complete Freund's adjuvant (CFA). On a weight basis this was equivalent to 0.913 μg of S region protein and 0.087 μg of pre-S(2) region protein per mouse or approximately a tenfold excess of S region protein. Ten days after immunization, all the strains in Fig. 1 contained immunoglobulin G (IgG) specific for the pre-S(2) region polypeptide but none for the S region polypeptide, even though they had received a tenfold greater dose of S region protein than of pre-S(2) region protein. The responses of these strains to a 4-μg dose of HBsAg/p25 are shown with broken lines for comparison (these lines represent re-

sults for a 46-fold greater dose of S region antigen compared to pre-S(2) region protein). At 10 days, the response to the pre-S(2) region was greater than the responses to the S region in each strain immunized with 4 μg of HBsAg/p25 (Fig. 1). At 24 days, in strain B10 the response to the pre-S(2) region was 25-fold greater than that to the S region (Fig. 1a), and in B10.D2 the response to the pre-S(2) region was 200-fold greater than that to the S region (Fig. 1b). Strain B10.S(9R) was still nonresponsive to the S region at 24 days, yet produced a 1:1620 titer of antibody to the pre-S(2) region at this time (Fig. 1c). At 24 days, the responses to the pre-S(2) region elicited by immunization with HBsAg/p34 were also higher than the responses to the S region after immunization with 4 μg of HBsAg/p25. The differences in magnitude between these two responses depended on the HBsAg responder status [B10.D2 > B10 > B10.S(9R), Fig. 1]. After secondary immunization the responses to the pre-S(2) and S regions were either equivalent [B10.D2, B10.S(9R)] or the pre-S(2) response was greater (B10, fourfold difference) (Fig. 1). This was not surprising in the B10.D2 strain, since immunization with HBsAg/p25 also induced a 1:40,000 titer in response to the S region (Fig. 1b). However, the quantity of antibody to the S region produced by the B10.S(9R) strain after secondary immunization with HBsAg/p34 (0.5 μg) was significantly greater (80-fold) than that produced after secondary immunization with 4 μg of HBsAg/p25 (Fig. 1c). The efficiency of the response to the pre-S(2) region in this strain suggested that this pre-S(2) response may have a positive influence on the S-specific response.

Immunization of the B10.S strain with HBsAg/p34 confirmed that the antibody response to the pre-S(2) region can stimulate the response to the S region. The B10.S strain is a total nonresponder to S region determinants even after secondary immunization with 4 μg of HBsAg/p25 [Fig. 2a, (*15*)]. The B10.S strain, however, produced an IgG response to the pre-S(2) region 24 days after immunization with HBsAg/p34 (Fig. 2a). Furthermore, upon secondary immunization with HBsAg/p34, this "S region–nonresponder" strain produced an anti-S–specific response (1:640) as well as a secondary anti–pre-S(2) response (1:5120). The antibodies to the S region produced by the B10.S and B10.S(9R) strains after HBsAg/p34 immunization were subtype-specific (for example, anti-HBs/*y*), whereas those produced by the B10 and B10.D2 strains were both group- and subtype-specific. The B10.BR strain was a low responder to the pre-S(2) region, but nonetheless it produced a superior antibody to the pre-S(2) region compared to the S-specific antibody it produced 24 days after HBsAg/p34 immunization and compared to the antibody to the S region it produced after HBsAg/p25 (4 μg) immunization (Fig. 2b). The B10.M strain was a virtual nonresponder to both the S and pre-S(2) regions of HBsAg (Fig. 2c). Cumulatively, these results indicate that the pre-S(2) region of HBsAg is a superior immunogen to the S region at the humoral level with respect to the effective immunization dose and the magnitude and the time of onset of the primary response. Furthermore, the pre-S(2) region can have a positive influence on the S region–specific response because immunization of an S region–nonresponder strain with HBsAg/p34 can circumvent nonresponsiveness to the S region.

Since the anti–pre-S(2) response was measured on HBsAg particles consisting of both pre-S(2) and S region determinants (HBsAg/p34), it was necessary to establish a pre-S(2)–specific assay. It had been reported that pre-S(2) region antigenicity was resistant to denaturation by reduction and detergent treatment (*10*). In contrast, the major group- and subtype-specific S region antigenic determinants are conformation-dependent and quite sensitive to denaturing conditions (*18, 19*). Therefore, we were able to use HBsAg/p34 particles treated with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (2ME) as solid-phase antigen to measure the activity of antibody to the pre-S(2) region but not the S region. The recently described synthetic peptide encompassing the 26 amino acid residues at the NH_2 terminus of the pre-S(2) region (*10*) was also used as a solid-phase ligand to assay antisera produced by immunization with the HBsAg/p34 particles.

As illustrated in Fig. 3, antisera to group-specific (anti-HBs/*a*) and subtype-specific (anti-HBs/*y*) determinants of the S region were reactive against HBsAg/

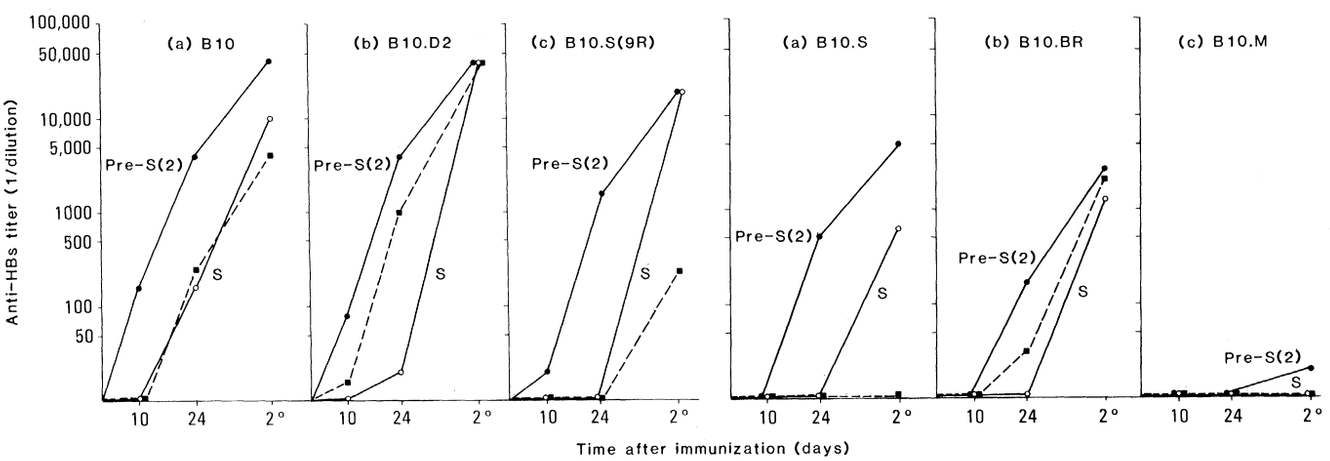

Fig. 1 (left). Comparison of production of antibodies to the S and pre-S(2) region of HBsAg in vivo. Groups of 5 mice of the indicated H-2 congenic strains were immunized intraperitoneally (i.p.) with 1 μg of HBsAg/p34 particles. The relative amount of p34 in the CHO-derived HBsAg particles was 35 percent (*17*), and the pre-S(2) region accounts for approximately 25 percent by weight of p34. Therefore, a 1-μg dose of HBsAg/p34 particles is equivalent to 0.913 μg of S region protein and 0.087 μg of pre-S(2) region protein. The IgG antibody responses specific for the S region (assayed with HBsAg/p25) and the S plus the pre-S(2) regions (assayed with HBsA/p34) were determined by solid-phase RIA (*14*) 10 and 24 days after primary immunization and 2 weeks after secondary immunization (2°). Antisera were also examined in a pre-S(2) region–specific assay (Fig. 3). For comparison the broken line represents the response of mice immunized intraperitoneally with 4 μg of HBsAg/p25, which lacks pre-S(2) region determinants. The HBs antibody titers are expressed as the highest dilution to yield 2.5 times the counts of preimmunization sera. Fig. 2 (right). Comparison of production of antibodies to the S and pre-S(2) regions. Groups of five mice of the indicated H-2 congenic strains were immunized and sera analyzed as described in Fig. 1.

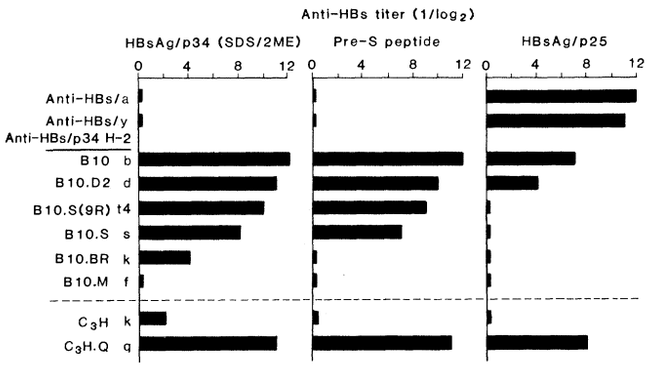

Fig. 3. Specificity and H-2 restriction of the antibody responses to the pre-S(2)region. H-2 congenic murine strains were immunized with 1 μg of HBsAg/p34 and 24-day sera were analyzed for IgG antibody specific for the pre-S(2) region of HBsAg/p34, a synthetic pre-S peptide (*10*), and HBsAg/p25 by RIA. A pre-S(2) region–specific RIA was developed utilizing solid-phase reduced and denatured HBsAg/p34 particles [HBsAg/p34 (SDS/2ME)]. HBsAg/p34 was treated with a final concentration of 2 percent SDS and 2 percent 2ME for 2 hours at 37°C, diluted in 0.01*M* bicarbonate buffer (*p*H 9.6), coated overnight on microtiter plates, and used in the standard RIA assay (*14*). This treatment was shown to reduce S region antigenicity by greater than 90 percent and did not effect pre-S(2) region antigenicity. The reactivities of group-specific (anti-HBs/*a*) and subtype-specific (anti-HBs/*y*) antisera to HBsAg/p25 are also shown. The HBs antibody titers are expressed as the reciprocal of the $\log_2$ of the highest dilution to yield 2.5 times the counts of sera before immunization. The H-2 haplotype of each strain is shown.

p25 particles but totally unreactive against HBsAg/p34 particles treated with SDS/2ME and against the synthetic pre-S peptide. This indicates the absence of cross-reactivity between S region antigens and pre-S(2) region antigens. A series of murine strains were immunized with HBsAg/p34, and the IgG antibody responses after 24 days were compared on the three solid-phase antigens shown in Fig. 3. The B10, B10.D2, and $C_3H.Q$ strains produced antibodies specific to both the pre-S(2) region [detected with HBsAg/p34 (SDS/2ME) and the synthetic peptide] and the S region (detected with HBsAg/p25) at 24 days; however, in all cases the pre-S(2) response was significantly greater. The quantity (titer) of pre-S(2) antibody detected in these strains with HBsAg/p34 (SDS/2ME) was virtually equivalent (no greater than a twofold difference) to that detected with the pre-S peptide. The B10.S(9R) and B10.S strains demonstrated a pre-S(2)–specific response after 24 days but no S-specific response at this time (Fig. 3). In the B10.BR and C_3H strains (both H-2^k), after primary immunization, pre-S(2)–specific antibody was detectable only with the HBsAg/p34 (SDS/2ME) particles and not with the pre-S peptide. This may represent a quantitative difference in these low responding strains since a response to the pre-S peptide was detected following secondary immunization. Alternatively, these strains may recognize a pre-S(2) region determinant not represented on the synthetic peptide.

To further explore the specificity of the antibody to the pre-S(2) region, we compared antiserum to the native pre-S(2) region with antiserum to the synthetic pre-S peptide using competitive antibody inhibition assays. The antiserum to the native pre-S(2) region was produced in B10 mice immunized with HBsAg/p34 and collected 10 days after immunization. This antiserum had no anti-S reactivity (Fig. 1a). The antiserum to the synthetic pre-S peptide was raised in rabbits as described elsewhere (*10*). As shown in Fig. 4a, the reaction between HBsAg/p34 and antiserum to the pre-S peptide (1:5000) was quantitatively inhibited by dilution with antiserum to the native pre-S(2) region whereas the control antiserum to the native S region had no inhibitory effect. In the reciprocal experiment, the reaction between HBsAg/p34 and antiserum to the native pre-S(2) region (1:500) was also quantitatively inhibited by antiserum to the pre-S peptide, and no effect was observed with a control antiserum to an S peptide (Fig. 4b). The failure to achieve 100 percent inhibition indicates recognition of distinct antigenic determinants by these two antisera; however, the significant competition observed indicates that antisera to the peptide and to the native pre-S(2) region also recognize overlapping determinants on the pre-S(2) region.

Antisera produced by immunization with native HBsAg/p34 recognized native and denatured HBsAg/p34 and the synthetic pre-S peptide. The quantity of pre-S(2) antibody detected with the solid-phase pre-S peptide was similar to that detected with solid-phase denatured HBsAg/p34. This represents the reaction of antibody to the native pre-S(2) region with a synthetic peptide at high efficiency and suggests that a large proportion of the response to the pre-S(2) region is directed to the 26 NH_2-terminal residues in the pre-S(2) region. Additionally, Western blot analysis of antiserum that was classified as pre-S(2) specific by radioimmunoassay (RIA) bound only the p34 polypeptide and not p25/gp28. Furthermore, the presence of pre-S(2) region determinants on HBV was confirmed by the observation that the pre-S(2)–specific antisera produced by immunization with HBsAg/p34 bound to 100 percent of 42-nm intact virions as analyzed by immunoelectron microscopy. The degree of identity between the native and peptide antisera and the resistance of native pre-S(2) antigen to reduction and denaturation are consistent with the presence of sequential-dependent as opposed to conformation-dependent determinants on the pre-S(2) region. This is in contrast to the major group- and subtype-specific determinants of the S region, which are highly dependent on intact disulphide bonds. In

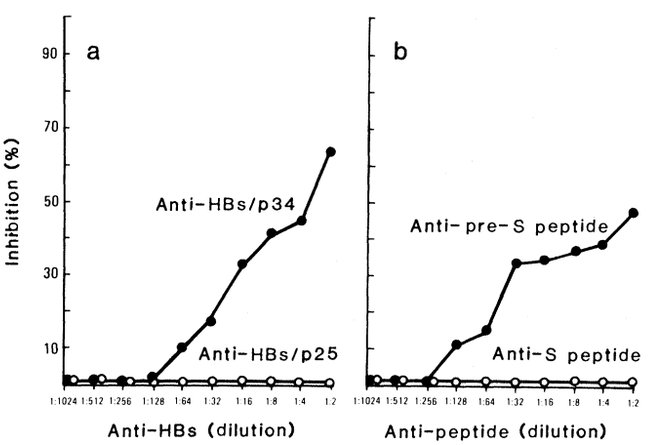

Fig. 4. Competitive antibody inhibition assays comparing antisera to the pre-S peptide and the native pre-S(2) region. (a) Solid-phase HBsAg/p34 was incubated with dilutions of mouse antibodies to the native pre-S(2) region overnight at 4°C. A limiting amount (1:5000) of rabbit antibodies to the pre-S peptide was added followed by goat antibodies to rabbit IgG labeled with ^{125}I, and the degree of inhibition was determined. (b) Solid-phase HBsAg/p34 was incubated with dilutions of rabbit antibodies to the pre-S peptide overnight at 4°C. A limiting amount (1:500) of mouse antibodies to the native pre-S(2) region was added followed by goat antibodies to mouse IgG labeled with ^{125}I, and the degree of inhibition was determined.

view of the enhanced immunogenicity of the pre-S(2) region, it is conceivable that sequential determinants in a globular protein may possess an inherent advantage in terms of immune cell recognition and promotion of efficient cellular interactions.

The quantity (Fig. 3) and kinetics (Figs. 1 and 2) of the production of antiserum to the pre-S(2) region in vivo were shown to vary from high-responder to nonresponder phenotype among the H-2 congenic strains examined. On the basis of these results we can classify the b, d, and q haplotypes of H-2 as high responders to the pre-S(2) region (10- and 24-day responses), the H-2^s haplotype as intermediate (24-day response), the H-2^k haplotype as low (marginal 24-day response), and the H-2^f haplotype as a nonresponder. Antibody production and T-cell proliferation that are specific for S region determinants are regulated by H-2–linked Ir genes (*15*). However, the response of the H-2 haplotypes to the S region differs from their response to the pre-S(2) region. For example, the H-2^d and H-2^q haplotypes are high responders to the S region, whereas the H-2^b haplotype is intermediate, and the H-2^k haplotype is a low responder but superior to the nonresponder H-2^f and H-2^s haplotypes. The H-2 regulation of the pre-S(2) response differs in that the H-2^b haplotype is at least equivalent to the H-2^d and H-2^q haplotypes, and the H-2^s haplotype is an intermediate as opposed to a nonresponder phenotype. These data indicate that distinct H-2–linked genes influence S- and pre-S(2)–specific antibody production in vivo.

The S and pre-S(2) regions of HBsAg are composed of 226 and 55 amino acid residues, respectively. This system raises several questions: will the antibody response to the pre-S(2) region appear 'hapten-like' and be regulated through T-cell recognition of S region determinants, or will it be regulated independently? Will an immune response to one region influence the immune response to the other? that is, will there be a carrier effect? Since we had previously mapped and determined the influence of the H-2–linked Ir genes that regulate the immune response to the S region of HBsAg (*15*), we were able to examine these questions with regard to the pre-S(2) region. Although distinct H-2–linked genes influence S- and pre-S(2)–specific antibody production, S and pre-S determinants exist on the same polypeptide (p34), and it is conceivable that helper T cells specific for determinants on one region may be capable of providing functional help to B-cell clones recognizing determinants on the other region. The enhanced immunogenicity of the pre-S(2) region and the predominance of IgG class antibodies to the pre-S(2) region as early as 10 days following primary immunization suggests efficient T-cell helper influence. Investigation of the T-cell proliferative responses to the pre-S(2) region revealed dramatic differences between pre-S(2) and S region immunogenicity at the T-cell level (*20*). The effective priming dose required to induce a pre-S(2)–specific proliferative T-cell response was lower than that required to induce an S-specific response. In addition, the overall magnitude of the pre-S(2)–specific responses was greater and the minimal antigen dose required to elicit T-cell proliferation in vitro was less for the pre-S(2) region than for the S region. The B10.S strain is nonresponsive to HBsAg/p25; yet, when immunized and boosted with HBsAg/p34, it produced an antibody response to the S region as well as to the pre-S(2) region. We have observed that this strain is nonresponsive to the S region but responsive to the pre-S(2) region at the T-cell level (*20*); therefore, it appears that pre-S(2) region determinants act as carriers for S region hapten in this strain. We have previously shown that the nonresponder status of the B10.S strain can be bypassed by immunization with a red blood cell–HBsAg/p25 conjugate (*21*). Since strain B10.S responded to the product of the pre-S(2) region but did not respond to that of the S region, it was possible to observe the influence of the pre-S(2) response on the S response.

Cumulatively, these results indicate the following: (i) the pre-S(2) region of HBsAg is significantly more immunogenic than the S region in terms of antibody production in vivo; (ii) the 26 amino acid residues at the NH_2-terminus of HBsAg/p34 represent a dominant antibody-binding site on the pre-S(2) region; (iii) production of antibodies to the pre-S(2) region of HBsAg is regulated by H-2–linked genes; (iv) H-2 restriction of the immune response to the pre-S(2) region is distinct from the H-2 restriction of the response to the S region; and (v) overlapping regulatory mechanisms exist since immunization with HBsAg/p34 of a strain that responds to the product of the pre-S(2) region but not to that of the S region can circumvent nonresponsiveness to the S region. These characteristics of the immune response to the pre-S(2) region are not unique to the recombinant particles derived from CHO cells since studies with serum-derived, pre-S(2) region–positive HBsAg and purified viral particles gave equivalent results (*22*).

These data have implications for the development of alternative HBsAg vaccines. The pre-S(2) region is a superior immunogen compared to the S region of HBsAg. H-2–linked genes regulate the responses to pre-S(2) and S region determinants independently, and therefore the likelihood of genetic nonresponsiveness to the entire particle is decreased. It should be noted, however, that one murine H-2 haplotype (H-2^f) is nonresponsive to both regions of HBsAg. Finally, the pre-S(2) region appears capable of generating T-cell helper activity that can function to induce production of antibodies to the S region as well as pre-S(2) region, thereby circumventing S region nonresponsiveness. These characteristics suggest that inclusion of the pre-S(2) region could augment the effectiveness of future HBV vaccines, depending on the ability of pre-S(2)–specific antibodies to protect against HBV infection as previously demonstrated for S region–specific antibodies (*23*).

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Serologic Identification and Characterization of a Macaque T-Lymphotropic Retrovirus Closely Related to HTLV-III

Abstract. *Human T-lymphotropic virus type III (HTLV-III) is thought to play an etiologic role in the development of the acquired immune deficiency syndrome (AIDS). In this study the serologic characterization of a new simian retrovirus that is related to HTLV-III is described. This new virus, here referred to as STLV-III, was isolated from sick macaques at the New England Regional Primate Research Center. Radioimmunoprecipitation analysis revealed STLV-III–specific proteins of 160, 120, 55, and 24 kilodaltons, all similar in size to the major* gag *and* env *proteins of HTLV-III. These antigens were recognized by representative macaque serum samples and human reference serum samples positive for HTLV-III antibodies. Monoclonal antibodies directed to p24, the major core protein of HTLV-III, also immunoprecipitated a 24-kilodalton species in lysates of cells infected with the macaque virus. This HTLV-III–related virus, which naturally infects a nonhuman primate species, may provide a useful model for the study of HTLV-III and the pathogenesis of AIDS.*

The human T-lymphotropic retroviruses (HTLV) are a group of related exogenous agents that preferentially infect helper T lymphocytes. There are three known types. HTLV-I is characterized by its widespread yet geographically distinct distribution; in endemic regions it has been closely linked with the development of a unique lymphoma of mature T cells designated the adult T-cell leukemia/lymphoma (ATLL) (*1*). HTLV-II is closely related to the type I virus, but its distribution and relation to human disease has not been established (*2*). HTLV-III, also known as LAV and ARV, is the prototype virus isolated from patients with the acquired immune deficiency syndrome (AIDS) (*3*, *4*). Thus, it appears that at least two members of this virus family are highly associated with different types of human diseases that include a malignancy and an ablative disorder of the same $T4^+$ lymphocyte population.

The existence of a virus related to HTLV-I in nonhuman primates was first reported by Miyoshi and his colleagues (*5*). Studies with healthy Japanese macaques, *Macaca fuscata*, indicated that various proportions of healthy adults (of this species) had antibodies that reacted with HTLV-I–related antigens. Type C retroviruses that were presumably responsible for this activity were then detected in lymphoid cultures established from seropositive animals (*6*). Subsequent serologic studies have demonstrated the presence of antibodies to HTLV-I in Asian and African Old World primate species, whereas New World primates and prosimians have been uniformly seronegative (*7*, *8*). Proviral sequence analyses of viruses derived from seropositive baboons, African green monkeys, and *Macaca* species indicate that the nonhuman primate viruses are closely related to, yet distinct from, HTLV-I (*9*).

We recently reported an association between exposure to an HTLV-I–related virus and the development of spontaneous lymphoma and lymphoproliferative disorders in three species of macaques. Antibodies highly cross-reactive with HTLV-I–related antigens were detected by means of membrane immunofluorescence (MIF) and radioimmunoprecipitation (RIP) techniques (*8*). Macaque sera

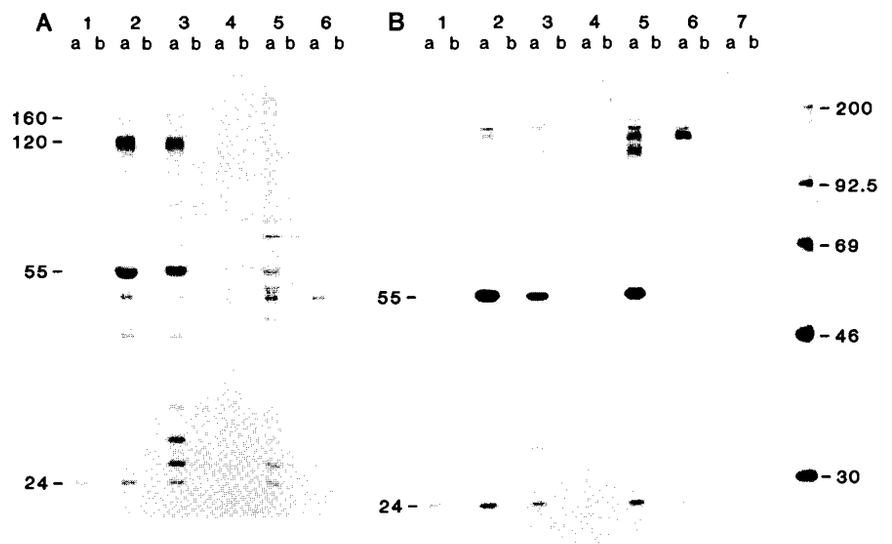

Fig. 1. (A) H9/HTLV-III and H9 cells were harvested at their peak of log-phase growth and were exposed to [^{35}S]cysteine [150 μCi/ml; specific activity, 1000 to 1050 Ci/mmole; New England Nuclear] for 8 to 10 hours. A soluble cell lysate was prepared by disruption of cells with RIPA buffer (0.15*M* NaCl, 0.05 tris-HCl, *p*H 7.2, 1 percent sodium deoxycholate, and 0.1 percent SDS) and centrifugation for 1 hour at 100,000*g*. The lysates [H9/HTLV-III (lanes a); H9 (lanes b)] were reacted with 10 μl of the following test sera bound to protein A–Sepharose CL-4B (protein A beads, Sigma): (Lane 1) Monoclonal antibody to HTLV-III p24; (lane 2) human reference positive serum to HTLV-III; (lane 3) human reference positive serum to HTLV-III; (lane 4) human reference negative serum to HTLV-III; (lane 5) representative macaque serum, positive for STLV-III; and (lane 6) representative macaque serum, negative for STLV-III. The immunoprecipitates were eluted in a sample buffer containing 0.1M Cleland's reagent, 2 percent SDS, 0.08*M* tris-HCl, *p*H 6.8, 10 percent glycerol, and 0.2 percent bromophenol blue by boiling at 100°C or 2 minutes. Samples were analyzed in a 10.0 percent acrylamide resolving gel with a 3.5 percent stacking gel according to the discontinuous buffer system of Laemmli (*19*). (B) HUT-78/STLV-III (lanes a) and uninfected HUT-78 (lanes b) cell lysates were prepared as described above. (Lane 1) Monoclonal anti-p24 HTLV-III; (lane 2) human reference positive serum to HTLV-III; (lane 3) human reference positive serum to HTLV-III; (lane 4) human reference negative serum to HTLV-III; (lane 5) representative macaque serum, positive for STLV-III; (lane 4) representative macaque serum, positive for STLV-III; and (lane 7) representative macaque serum, negative for STLV-III.