Immune Response of Monkeys to Lymphotrophic Herpesvirus Antigens

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Summary

Herpesvirus saimiri induces leukemia and/or malignant lymphoma when inoculated into different species of nonhuman primates including marmosets and owl monkeys. No malignant disease, however, has been recognized in the natural host for this virus, the squirrel monkey, although a high percentage of these monkeys are chronically infected with H. saimiri. Furthermore, one species of marmosets, as well as capuchin and some owl monkeys, fail to develop lymphoma following experimental virus infection but developed a chronic infection similar to that noted in the natural host. The availability of susceptible and resistant species made it possible to attempt to delineate those humoral and cellular immune parameters that might correlate both with tumor induction and resistance. The findings from these investigations are reviewed and discussed in relation to vaccine studies in this system as a model for a human herpesvirus vaccine.

Introduction

HVS\(^3\), isolated in 1968 by Melendez et al. (12) from a degenerating squirrel monkey kidney culture, induces leukemia and/or malignant lymphoma when inoculated into a variety of nonhuman primates including owl monkeys and marmosets (1, 5, 8, 11, 13, 32). Lymphoma induction also occurs in rabbits following infection with this virus (3). The neoplastic disease induced by HVS in primates has the characteristics of a T-cell lymphoma since the target cell for HVS has been shown to be the T-lymphocyte (30). Not all species of primate or all animals in a given species, however, develop lymphoma when infected with this virus. No malignant disease has been recognized in its natural host, the squirrel monkey, although a high percentage of these monkeys are chronically infected as determined by virus isolation studies and serological analysis (5). Furthermore, one species of marmosets as well as capuchin and some owl monkeys failed to develop lymphoma following experimental virus infection (10, 18, 19, 24).

The availability of susceptible and resistant species made it possible to attempt to delineate those humoral and cellular immune parameters that might correlate both with tumor induction and resistance. The findings from these investigations have resulted in the definition of certain immune response that appear to be indicators of disease induction as well as others that might play an active role in immunity to disease induced by the virus.

Antibody Responses to HVS-associated Antigens in Susceptible and Resistant Species

A number of virus-associated antigens have been defined in HVS-infected cells primarily by immunofluorescence techniques. These antigens were designated LA for cytoplasmic antigens produced late in the virus replication cycle; EA for nuclear antigens synthesized in the presence of inhibitors of DNA synthesis; and MA for HVS-associated membrane antigens also produced late in the virus replication cycle (9, 15). Blocking tests established that EA and LA were distinct antigenic entities (9). The relationship of MA to viral envelope antigens has not been resolved conclusively. However, preliminary findings suggest that MA may not be fully expressed in the viral envelope (H. Rabin and G. Pearson, unpublished findings) as appears to be the case with other herpesviruses such as the Epstein-Barr virus, herpes simplex, and the Marek's disease herpesvirus (2, 16, 17, 27).

Extensive analysis of sera from resistant and susceptible species has revealed some interesting differences in the antibody responses to these groups of antigens. Most naturally infected squirrel monkeys have antibodies to LA and MA as well as neutralizing antibodies but rarely have antibodies to EA (9, 23). Following the initial exposure of young squirrel monkeys to HVS, antibodies to all 3 groups of immunofluorescent antigens were produced and were initially detected at approximately the same time (9). Antibodies to EA, however, did not persist but gradually decreased to undetectable levels over an 8-month observation period. Antibodies to the other groups of antigens persisted at fairly constant levels. The anti-EA response was completely different in those animals susceptible to lymphoma induction by HVS. In marmosets and owl monkeys that developed leukemia and/or lymphoma following HVS infection, the antibody response to this particular group of antigens generally followed a disease-related pattern (9, 18). Antibody induction usually was delayed in comparison to the antibody responses to LA and MA and usually preceded or coincided with the development of malignant disease. Furthermore, antibody titers tended to increase with disease progression.

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1. Presented at the symposium: Immunological Control of Virus-associated Tumors in Man: Prospects and Problems, April 7 to 9, 1975, Bethesda, Md.
2. Presenter.
3. The abbreviations used are: HVS, Herpesvirus saimiri; T, thymus derived; MF, membrane immunofluorescence; ADLC, antibody-dependent lymphocyte cytotoxicity.
In some owl monkeys that did not develop lymphoma, with a few exceptions that will be discussed below, antibodies to EA were not produced although antibodies to LA and MA were present in moderate titers (18). These findings suggested that the antibody response to EA was a measure of tumor load. Further investigations indicated that increasing EA titers reflected the presence of increasing numbers of virus genome-carrying cells in the peripheral circulation of infected animals (19). Whether these virus-containing cells represent transformed cells is still unknown.

Interestingly, the antibody response to the EA complex in capuchin monkeys following HVS infection was intermediate between that noted in the natural host and in monkeys that developed malignant disease. In this species, a chronic virus infection similar to that noted in squirrel monkeys usually developed (24). In contrast to the findings in squirrel monkeys, however, antibodies to EA persisted at low to moderate levels in the absence of malignant disease over the entire observation period which with some of these animals lasted as long as 2 years. Two owl monkeys that did not develop lymphoma following virus infection showed a similar antibody response pattern over a prolonged observation period (18, 19). Low to moderate levels of virus were continuously rescued from all of these chronically infected monkeys, however, which might account for the persistence of antibodies to EA in the absence of apparent malignant disease. In general, however, the results from these serological investigations in resistant and susceptible monkeys indicated that increasing antibody titers to EA were indicative of disease.

To determine whether any of these humoral factors might be active in immunity to the virus, experiments were designed to determine whether antibody directed against HVS-associated MA could mediate the destruction of virus-infected or transformed cells. None of the sera from owl monkeys infected with HVS, which contained high antibody titers by MF, were cytotoxic in the presence of complement to cells expressing HVS antigens (H. Rabin, personal communication). Interestingly, however, these sera were effective in mediating the destruction of HVS-infected cells in vitro by normal peripheral blood lymphocytes (22). This in vitro cytotoxic reaction designated ADLC was originally described by Perlmann et al. (20) in xenogeneic and allogeneic systems and involves the interaction of antibody or antibody-antigen complexes with lymphoid cells bearing Fc receptors. ADLC has now been demonstrated against cells expressing C-type and herpesvirus antigens (6, 7, 21, 26, 28, 29). The significance of this reaction in vitro, however, is still unknown although there have been some published reports that suggest that this immune mechanism might indeed be active in vivo in the destruction of virus-infected or transformed cells expressing MA (6, 7). Surprisingly, however, the preliminary findings in the HVS system indicated that the antibody titers to MA as determined by the ADLC assay were significantly higher in owl monkeys that developed lymphoma than in those monkeys that showed no disease manifestations and that were presumably immune (22). These findings differ from those reported from investigations in C-type virus systems where ADLC titers generally correlated with immunity (6, 7). The reason for this difference is unknown although it might reflect a difference between those viruses such as C-type viruses that continue to replicate both in the transformed and normal cells of the diseased host thereby producing excessive amounts of antigen and those viruses such as herpesviruses where antigen expressing in vivo is minimal. Further studies are needed to determine the importance of this cytotoxic reaction mediated by antibody directed against MA in resistance to HVS infection.

**Cellular Immunity Studies**

Investigations were undertaken to determine whether an active cellular immune response against HVS-associated antigens could be demonstrated in resistant monkeys. This was a particularly important question in this system since the target cell for HVS in the T-lymphocyte (30) and previous studies had demonstrated that, in general, lymphocytes from virus-infected owl monkeys became unresponsive to T-cell mitogens with the development of malignant disease (31).

Initial experiments using a variety of in vitro assays failed conclusively to detect a specific active cellular immunity in chronically infected animals (W. Wallen and G. Pearson, unpublished findings). Recently, however, the isotopic footpad assay originally described by Paranjpe and Boone (14) was used to attempt to demonstrate cellular immunity to HVS antigens by the transfer of local adoptive immunity from monkey to mouse. This assay was chosen since preliminary experiments in another system suggested that this cellular immunity test was applicable for the demonstration of xenogeneic transfer of local adoptive immunity (4). In experiments designed to detect cellular immunity to HVS-associated antigens, 10 x 10^6 lymphocytes from normal or infected monkeys were mixed with 3 x 10^6 cells from an HVS-transformed marmoset cell line designated MLC (25), and the mixtures were inoculated into the right footpad of normal BALB/c mice. An Epstein-Barr virus-transformed cell line (229) isolated from an inoculated marmoset served as a control antigen in one experiment. Immediately thereafter, 125I-labeled human serum albumin was injected i.p. Twenty-four hr later, the inoculated test footpad and the contralateral control footpad from each mouse were removed at the junction of the lower and middle third of the tibia and counted in a γ spectrometer. Results are expressed as the foot count ratio, i.e., cpm from the test footpad divided by cpm from the control footpad.

Results from 2 separate experiments using this assay are presented in Charts 1 and 2. The results from 1 experiment using splenic lymphocytes from normal and HVS-infected squirrel monkeys against MLC and 229 cells are shown in Chart 1. Although immune splenic lymphocytes produced significant reactions against both target cells in comparison to normal lymphocytes, the reactions were significantly greater with MLC than with 229 suggesting some specificity in this test. A similar trend was noted with lymphocytes from 1 chronically infected owl monkey and from 3 infected capuchin monkeys (Chart 2). Significant activity against MLC cells was noted with lymphocytes again suggesting...
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![Graph](chart1.png)

Chart 1. Local adoptive transfer of immunity to HVS-associated antigens by splenic lymphocytes from naturally infected squirrel monkeys as determined by the isotopic footpad methods. Each point (●) represents the footpad ratio from 1 inoculated mouse; —, mean value for each group. Significance of differences between mean values for each group was determined by Student's t test.

![Graph](chart2.png)

Chart 2. Local adoptive transfer of immunity to HVS-associated antigens by peripheral blood lymphocytes from capuchin and owl monkeys experimentally infected with HVS as determined by the isotopic footpad methods. Each point (●) represents the footpad ratio from 1 inoculated mouse; —, mean value for each group. Significance of differences between mean values for each group was determined by Student's t test.

immune recognition of a virus-associated antigen. The results from these experiments, although very preliminary, suggest that an active cell-mediated immunity directed against HVS-induced antigen might exist in chronically infected monkeys and therefore could be responsible for inhibiting lymphoma production. Further experiments with larger groups of animals and the use of more control materials are required, however, before conclusions can be drawn concerning the specificity of the cellular reaction detected with this assay.

Conclusions

The choice of an appropriate animal model system for virus vaccine studies depends to a large extent on how well the biological aspects of the virus-host interactions have been defined. This is necessary for appropriate evaluation of both the immunogenicity and effectiveness of a given vaccine preparation. From this point of view, HVS deserves serious consideration as a model for herpesvirus vaccine studies since the extensive immunological studies with this virus, reviewed in this paper, have defined a number of immune parameters that appear to reflect the course of the virus infection and that would be useful parameters for monitoring the effectiveness of a vaccine in preventing the disease manifestations induced by HVS. The most significant of these responses appears to be the antibody response to the HVS-associated EA complex. This antibody response is closely related to the presence of malignant disease in the infected animal, although this relationship has not been without exceptions. There have been a few infected animals that have developed persisting levels of antibody to EA in the absence of apparent malignant disease as discussed earlier in this paper. However, these monkeys have shown other signs of an active infection that could account for the persisting anti-EA titers. The preliminary findings on the ADLC antibody response suggest that it might also follow a disease-related pattern. Antibody titers determined by this assay appeared to be significantly higher in those monkeys that developed malignant disease than in resistant animals. Interestingly, antibody titers to HVS-MA determined by MF were not significantly different in resistant and susceptible animals (22) suggesting that humoral factors other than the antibody measured by the MF test were capable of mediating ADLC and were present primarily in the sera of monkeys with malignant disease. The nature of these factors is not known although they could include antigen-antibody complexes since it has been reported that complexes can mediate ADLC (20). Alternatively, the ADLC test might detect an antibody species not detected in the MF assay.

The role of the cellular immune response in resistance to HVS infection has still not been clearly defined. The preliminary findings presented in this paper, however, using the isotopic footpad assay for detecting the xenogeneic transfer of local adoptive immunity from monkey to mouse, have provided some evidence suggesting that an active cellular response specific to HVS-associated antigens may be present in naturally or chronically infected monkeys. Further studies, however, are required to substantiate these initial findings. However, if these results can be confirmed in larger groups of animals, this assay would be applicable for monitoring the cellular immune response of monkeys inoculated with a vaccine thereby providing a herpesvirus model system where the immunogenicity of a given vaccine preparation could be readily monitored by both humoral and cellular immunological assays.

References

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