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Serologic Surveys for Viral Antibodies in Cancer Patients[1]

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To the epidemiologist it is essentially a truism that proof of the viral etiology of a human tumor will come from the application of epidemiologic methods. That is, such proof must include demonstrating a correlation between experience with a particular virus and the risk of developing a certain type of tumor. Since in most laboratory model systems tumors caused by DNA viruses do not contain infectious virus, seroepidemiologic studies are almost essential for seeking such correlations.

The discovery of the oncogenicity of adenovirus (18) has resulted in the feeling that any virus can be considered a possible tumor virus; consequently, seroepidemiologic surveys against prevalent viruses, particularly various adenoviruses, have become of immediate importance. Much effort is being devoted by a number of laboratories to the problem of the type of serologic testing and the type of antigen that may yield useful results.

If there were a virus-specific, tumor-specific antigen, meaning a virus-coded antigen found only in tumors, the problem would be relatively simple. With the discovery of T antigens (7) and virus-specific transplantation antigens in viral tumors (3, 17), it appeared at first that such might be the case; however, T antigens have been found to represent a previously unrecognized class of viral proteins formed early in the normal replicative cycle (4, 13, 16), certain ones of which are produced in tumor cells. The limited evidence available indicates that the same is true of transplantation antigens (V. Defendi, personal communication). Thus, at present there is no viral antigen which can be considered unique for tumor cells.

The virologist tends to think of the antibodies detected in different serologic tests as being in different relationship to the time of the antigenic stimulus. Thus, in simplest form, neutralizing antibody is considered highly "durable," reflecting infection with a virus at any previous time in the life of the individual; complement-fixing (CF) antibody is less durable and tends to indicate more recent experience and, on occasion, persistent infection; and the hamster tumor models have suggested that antibody to T antigens (at least as detected in the CF test) reflects current antigenic stimulation, since T antibody level often drops rapidly on removal of the tumor (5).

Seroepidemiologic studies of the incidence of neutralizing antibodies in various types of cancer patients have the advantage of being based on minimal assumptions about the type of virus-tumor relationship but have a great disadvantage because of the difficulty of detecting excess antibody incidence in the face of a high incidence of antibody in the controls. Because of this problem, surveys for neutralizing antibody to Ad. 12 and 18 (R. R. Rafajko, R. J. Huebner, and A. M. Lewis, Jr., unpublished data) in cancer patients have given little meaningful information, other than that these are prevalent viruses of the general population.

Surveys of CF antibodies to classical viral structural antigens are primarily testing the hypotheses that recent infection (within a year or two) led to the development of tumor, or that the tumors are producing this type of antigen. Epidemiologic studies of human cancer and animal model systems suggest that both of these hypotheses are rather unlikely. The greatest chance of detecting correlation between tumor occurrence and recently formed antibody would be in studies of patients with precancerous changes or *in situ* malignancy. Because of this possibility, several years ago we assisted in a small controlled study of CF antibodies to various common viruses in sera of patients with such lesions of the uterine cervix (9). Although the results were negative, the type of approach is worthy of further consideration.

The most hopeful approach to serologic surveys is, of course, based on the T antigen model. To date, CF testing of human sera (either from patients with acute adenovirus infections or from cancer patients) against adenovirus hamster tumor extracts and T antigen preparations from acutely infected tissue culture cells, as well as testing of human tumors against hamster T antibody has given essentially negative results (8, 14, 15) (R. J. Huebner and A. M. Lewis, Jr., unpublished data; H. W. Kim, R. H. Parrott, and R. M. Chanock, personal communication; J. J. Trentin, personal communication). A low incidence of reactions has been observed in both directions, but a similar incidence has been obtained with control patient material, and the possibility that these represent isoantigen reactions has not been eliminated.

In our experience, the indirect fluorescent antibody (FA) technic has been more sensitive than CF for detection of T antibodies in animal systems (1, 10), and with this technic, antibodies reactive with adenovirus T antigens have been found to be common in humans (10). The majority of reactions are obtained with T antigens formed during acute infection of tissue culture cells, but staining of hamster tumor cells has also been obtained.

Design and interpretation of seroepidemiologic studies with the adenovirus T antigen FA system is markedly hindered by the complexity of the antigens. There are at least 30 distinct adenoviruses of man, divisible into a number of subgroups, and at least 9 serotypes belonging to three of the subgroups have some degree of oncogenic potential (2, 6, 11). Each

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adenovirus produces a number of T antigens, with a variety of morphologic appearances in the FA test, some of which seem to be specific for the subgroup, while others are cross reactive with certain viruses of other subgroups (1, 12).

This diversity of antigens might not constitute such a problem were not antibody relatively common. In the limited seroepidemiologic studies which we have done to date, 10 to 50 percent of cancer patients have shown FA-stainable T antibody to any single adenovirus type tested; controls (hospitalized patients with nonmalignant disease), have given comparable results (A. M. Lewis, Jr., W. H. Wiese, and W. P. Rowe, unpublished data). This finding suggests either that the T antibodies in man are far more durable than in the tumor-bearing hamster or that half of our controls are harboring a focus of adenovirus antigens; it seems probable that the former hypothesis is the true one.

The problem might be greatly simplified by screening sera only against adenovirus-induced rodent tumor cells, since they probably contain a less broad spectrum of T antigens than do acutely infected cells. This should be done, but a negative epidemiologic result might just be the result of a different group of T antigens being expressed in human tumor cells than in the rodent.

Another serologic approach based on the T antigen model, which is logically appealing but in practice still without positive results in the case of solid tumors, is the testing of autologous serum on tumor cells grown in tissue culture (8, 14). (F. Bang, and S. R. S. Rangan, personal communication). More intensive study in this direction might yield a tumor cell antigen preparation containing a virus-specific antigen, which would be the best type of reagent for seroepidemiologic studies.

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