Avian Leukosis Antibody Response in Individuals Given Chicken Embryo Derived Vaccines (36117)

ALAN VE RICHMAN, CALVIN G. AULISIO, WILLIAM G. JAHNES, AND NICOLA M. TAURASO

U. S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Division of Biologics Standards, Laboratory of Virology and Rickettsiology, Bethesda, Maryland 20014

Under natural conditions, avian leukosis viruses (ALV) produce in chickens a variety of malignant disorders, manifested as solid invasive tumors, lymphomatosis, myeloblastosis, etc. As these agents are ubiquitous in conventional chicken flocks and are transmitted vertically from hen to egg, virtually all vaccines derived from conventional eggs are contaminated with ALV (1–4).

Experimentally, some strains of Rous sarcoma virus (RSV) have produced tumors in nonavian species (5), including nonhuman primates (6). Because of this, the question of the potential oncogenicity of ALV to humans arises.

There have been isolated reports of serologic surveys of ALV antibody in man (2, 3). These previous studies were limited to the detection of neutralizing antibody to the Bryan strain of RSV, a test only sensitive to subgroup A avian leukosis viruses.

We have, therefore, examined multiple sera from individuals inoculated with ALV-contaminated vaccines for the presence of antibody by employing the broadly reacting COFAL (7) and the highly sensitive indirect immunofluorescence (FAB) tests. The COFAL test is able to detect antibody to ALV of any subgroup. In the FAB test, subgroup A, B, and C antigens were employed to broaden the antigenic spectrum to cover the three largest ALV subgroups. Neutralization tests were performed on some sera. The results of these studies constitute this report.

Materials and Methods. Vaccines. All ALV-contaminated vaccines were produced in chicken embryos derived from conventional flocks and were known to be contaminated with ALV. The ALV-free yellow fever vaccine (YFV) was prepared in ALV-free eggs from seed lots also free from ALV (4).

Sera. Pre- and postimmunization serum samples were obtained from the following individuals inoculated with ALV-contaminated vaccines: 35 volunteers who received formalin-inactivated influenza vaccine in single or multiple doses; 15 inoculated with formalin-inactivated epidemic typhus vaccine; 15 who were given live attenuated measles vaccine; 15 who received conventional 17D YFV; and 15 who were given live smallpox vaccine. In addition, 200 pairs of pre- and postimmunization sera were obtained from individuals who received YFV: 25 from each of 4 groups, each receiving different lots of conventional ALV-contaminated vaccine and 25 from each of 4 groups, each receiving 4 different lots of ALV-free YFV in the same study. These sera were examined for the presence of group-specific COFAL and subgroup-specific FAB antibodies. In addition, sera from 7 laboratory personnel after multiple vaccinations with killed influenza, epidemic typhus, Rocky Mountain spotted fever, Q fever, and Japanese encephalitis vaccines and with live yellow fever vaccines and 20 "paired" sera from children receiving ALV-contaminated live measles vaccines were examined for the presence of ALV neutralizing antibody.

Antigens. The COFAL positive antigen consisted of 20× concentrated chicken embryo fibroblasts (CEF) infected with the RPL 12-L37 strain of avian lymphomatosis virus, originally provided by W. Okazaki,

Regional Poultry Laboratory, East Lansing, Michigan. The fourth passage of virus-infected cells was suspended in one-twentieth the original volume of medium, and frozen and thawed three times in an acetone–dry ice bath before storage at —60°. Avian leukosis virus-free CEF, prepared similarly, served as the negative control in the COFAL test.

Three strains of RSV were employed in the indirect FAB test: RSV-RAV-1 (subgroup A), Schmidt-Ruppin (subgroup B) and Prague (subgroup C) viruses. These viruses were obtained through the Resources and Logistics Segment of the Special Virus Cancer Program of the National Cancer Institute. Infected cover slips were prepared for each virus according to previously described methods (8).

COFAL test. The complement-fixation (CF) procedure used in our laboratory has been described (9). Five 50% hemolytic units of complement and 8 units of antigen were added to serial 2-fold dilutions, starting with 1:5, of heat-inactivated (56° for 30 min) sera; the hemolytic system was added after incubation at 4° for 18 hr; and the mixture was incubated at 37° for 30 min with shaking of the plates at 10 and 20 min. Positive controls were included in each test.

Fluorescent antibody (FAB) test. Virus-infected and uninoculated control cover slips of CEF were flooded with a 1:5 dilution of serum and incubated at room temperature (22–25°) for 30 min. Subsequent washings, staining, mounting, and examination of the cover slips were essentially as previously described (8). Selected pairs of sera were absorbed with acetone-treated calf liver powder for 1 hr at 37°. The absorbed sera were tested at 1:5 dilution with, and without, guinea pig complement after incubation at 37° for 30 min and overnight. Positive controls were included in each test.

Neutralization tests were performed according to methods described by Rubin *et al.* (10).

Results. Virtually 100% of the vaccinees developed a significant antibody response to their respective immunogen. Two of 15 persons receiving a single dose of influenza vaccine had COFAL titers of 1:10 and 1:5 in their pre- and postimmunization serum samples, two receiving typhus vaccine had COFAL titers of 1:20 and 1:5 in both pre- and postsera, and two of 20 individuals receiving multiple doses of influenza vaccine for the preceding 10 to 20 years had COFAL titers of 1:20 in both pre- and postsera. All other sera were negative for COFAL antibody, and all sera were negative by the neutralization and the more sensitive FAB tests.

Discussion. We sometimes have difficulty interpreting COFAL tests because of low antibody titer, reactions between test sera and negative control antigen, and varying degrees of anticomplementary activity in some sera. Since the *few* COFAL positive titers were the same in both pre- and postimmunization sera, the "antibody," if specific, was certainly not related to the present immunization. Because the FAB test is considerably more sensitive and since this was uniformly negative in all serum samples, it was concluded that the COFAL reactions were most likely false positives. It should be noted, however, that specific CF reactions have not always coincided with positive immunofluorescence.

Although the titer of ALV was not determined in any of the vaccines used in this study, we assume that the titer was high, in the range of 10^5 to 10^6/ml. Other ALV-contaminated vaccines which we have examined had ALV titers in this range. In spite of the presence of ALV in the vaccines, none of the vaccinees developed ALV antibody. Our data corroborate the lack of immediate infectivity of ALV to man. However, the incidence of neoplasia has increased dramatically in recent years and the possibility of new cancer inducing and/or promoting agents in the environment or otherwise must be considered. It is theoretically possible, therefore, that the response of humans recently exposed to potential oncogenic viruses might be modified, and it is certainly plausible that these viruses may turn out to be more than innocent passengers. Recently, Roth and Dougherty (11) reported that there was no evidence of ALV group-specific antibodies in 101 human sera from leukemic patients. Nevertheless, it would therefore seem prudent to

maintain careful vigilance of persons exposed to known oncogenic viruses and to continually insure that vaccines not contain viruses having an oncogenic potential.

We have demonstrated in our laboratory that ALV-contaminated YFV can produce malignant lymphomatosis in chickens (unpublished observations). Therefore, it appears that simultaneous exposure to live attenuated yellow fever virus does not mollify the oncogenic effect of ALV in chickens.

To date no association has been found between cancer in man and immunization with vaccines containing the oncogenic ALV. From a retrospective study of large numbers of World War II veterans receiving ALV-contaminated YFV, no increased incidence of leukemia or other malignancy had been noted through 1967 (12). Although the latent period for oncogenic viruses in man is unknown, it may be argued that this should be a sufficient latent period for a proposed carcinogen to manifest itself.

1. Harris, R. J. C., Dougherty, R. M., Biggs, P. M., Payne, L. N., Goffe, A. P., Churchill, A. E., and Mortimer, R., J. Hyg. **64**, 1 (1966).

2. Markham, F. S., and Levine, S., Arch. Gesamte Virusforsch. **16**, 305 (1965).

3. Piraino, F., Krumbiegel, E. R., and Wisniewski, H. J., J. Immunol. **98**, 702 (1967).

4. Tauraso, N. M., Spector, S. L., Jahnes, W. G., and Shelokov, A., Proc. Soc. Exp. Biol. Med. **127**, 1116 (1968).

5. Vogt, P. K., *in* "Advances in Virus Research" (K. M. Smith and M. A. Lauffer, eds.), Vol. 11, p. 294. Academic Press, New York (1965).

6. Deinhardt, F., Nature (London) **210**, 443 (1966).

7. Sarma, P. S., Turner, H. A., and Huebner, R. J., Virology **23**, 313 (1964).

8. Aulisio, C. G., and Shelokov, A., Proc. Soc. Exp. Biol. Med. **126**, 312 (1967).

9. Tauraso, N. M., and Shelokov, A., Proc. Soc. Exp. Biol. Med. **119**, 608 (1965).

10. Rubin, H., Cornelius, A., and Fanshier, L., Proc. Nat. Acad. Sci. U.S.A. **47**, 1058 (1961).

11. Roth, F. K., and Dougherty, R. M., J. Nat. Cancer Inst. **46**, 1357 (1971).

12. Melnick, J. L., Nat. Cancer Inst. Mongr. **29**, 337 (1968).

Received Sept. 20, 1971. P.S.E.B.M., 1972, Vol. 139.