Immunologic Cross-Reaction Between Human Leukemic Plasma and Avian Leukosis Group-Specific Antiserum (35147)

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The avian leukosis viruses (ALV) belong to a diverse group of RNA viruses which shares common physicochemical characteristics as well as antigens common to all members of the group (1). Thus, hamsters bearing tumors induced by a given strain of Rous sarcoma virus (RSV) produce antibodies which react with all other strains of RSV as well as with other members of the ALV complex (2). The group has both a broad oncogenic spectrum and host range, being capable of producing solid tissue tumors, leukemias, and lymphomas in amphibians, reptiles, and mammals, including primates lower than man (3–5).

This paper reports the finding of antigens in the plasma of a patient with acute monomyelogenous leukemia which react in immunodiffusion tests with anti-serum from hamsters bearing RSV-induced tumors. In addition, it describes virus-like particles in the leukemic plasma which were demonstrated by electron microscopy.

Materials and Methods. Preparation of antisera. Tumor tissue originally transformed by the Schmidt-Ruppin strain of RSV was obtained from Dr. W. Okasaki, U. S. Poultry Research Laboratories, East Lansing, Michigan. Golden Syrian hamsters, 9–14 weeks old, were inoculated subcutaneously at a single site on the lower back with 0.5–1.5 mg of such tumor tissue. Histologically typical fibrosarcomas developed within 2 weeks of implantation and resulted in the death of the animals within 7 weeks. Blood was obtained from the retro-orbital sinus at various times after appearance of well-developed tumors, and the serum was separated and stored at 0° until tested for presence of antibodies against avian leukosis antigens.

Preparation of virus. Avian myloblastosis virus (AMV) was used as a control antigen for detecting the presence of avian leukosis group-specific antibodies in the sera of tumor-bearing hamsters. Blood of AMV-infected chickens was drawn at the time of terminal myeloblastosis. Virus was obtained from separated plasma by differential centrifugation as described by Bonar *et al* (6).

Serological procedures. Double-diffusion analyses of antigen/antibody systems were carried out in petri dishes containing 0.5% Ionagar No. 2. Wells (5 mm in diameter) were cut in the agar with an auto-gel cutter so that each was 5 mm distance from adjacent and center wells, respectively. All immunodiffusion patterns were read after 24-hr incubation in a moist chamber at room temperature.

Electron microscopy. Plasma (0.05 ml) was spread over the surface of 1.5% Bacto agar blocks measuring 1.25 mm^2. The specimens were floated from these blocks by collodion pseudoreplication onto 0.25% uranyl magnesium acetate and picked up on 400-mesh Cu grids. A thin coat of carbon was evaporated over the surface of each grid in a turbomolecular-pumped evaporator and the specimens observed in a turbomolecular-pumped RCA EMU-3H electron microscope at an instrumental magnification of 36,000 diameters (7). Estimates of particle concentration were based upon counts of 10 fields at an instrumental magnification of 12,000 diameters (8, 9).

Patient study. Patient P.R., a 13-year-old female, was in good health until 1 week prior to admission to Children's Hospital of Michigan. On admission a peripheral blood smear revealed a WBC of 81,000/mm^3 with 85% immature bizarre monocytoid cells. Examination of bone marrow showed 99% replacement by blastoid monomyeloblasts. On the second hospital day the WBC rose to 238,-

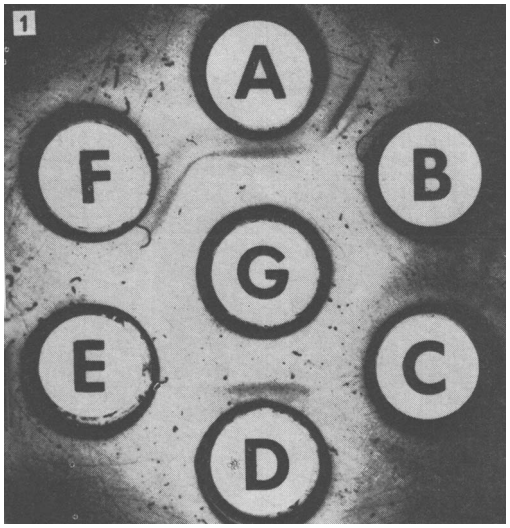

FIG. 1. Immunodiffusion reactions between: (A) serum from terminal tumor-bearing hamster No. 1, (B) AMV pellet from infected chicken plasma, (C) normal hamster serum, (D) serum from terminal tumor-bearing hamster No. 2, (E) normal human serum, (F) serum from terminal tumor-bearing hamster No. 3, and (G) plasma from patient P.R.

000/mm^3. A few hours before death on the third hospital day, blood studies revealed a WBC of 420,000/mm^3. The studies to be reported were performed on the plasma obtained from this terminal heparinized blood specimen.

Results. As seen in Fig. 1, P.R. plasma gave at least two precipitin bands with the serum of hamsters bearing viral-induced tumors. One of these bands is seen to merge in a reaction of identity with the precipitin bands formed by AMV and serum of tumor-bearing hamsters. The relationship of the second band to the AMV system is difficult to delineate by the immunodiffusion technique. It is important to note that P.R. plasma did not react with normal hamster serum, normal chicken plasma, or antinormal chicken serum in immunodiffusion tests. The plasma of 22 untreated leukemia patients, 25 leukemia patients in various stages of therapy, 60 normal adults, 7 normal children, and 45 syphilitic adults gave uniformly negative results when used in immunodiffusion tests against either normal or tumor-bearing hamster serum.

The results of the electron microscope studies on P.R. plasma as well as on control normal human plasma are complicated by the presence of a heavy background of finely textured plasma constituents (Figs. 2 and 3). However, the P.R. plasma contained "virus-like particles" measuring 46–56 nm (Fig. 2b) which were not seen in any of the normal plasmas studied. The concentration of these particles was estimated to be greater than 5×10^7 particles/ml based upon direct counts. About 20% of these particles appeared to be empty or "donut forms" (Fig. 3).

Discussion. The discovery of the presence of structural viral antigens in Rous sarcoma virus-caused tumors by Zilber (10) has prompted the search for such antigens in other animal and human tumors. When an avian leukosis virus causes transformation in avian species, both the virus and transformed cells survive and infectious virus is produced in all subsequent daughter cells. In this system both the type-specific viral envelope antigens and group-specific (GS) internal structural antigens are produced (11). However, when ALV is used to induce tumors in mammals, only the GS antigens are produced. Since the serum used as a source of antibodies in this study was from hamsters bearing RSV-induced tumors, the reactions in immunodiffusion tests must be between GS antigens present in AMV and antibodies against those antigens present in the hamster serum. The fact that this human plasma gave two precipitin lines, one of which definitely merged with those due to the AMV anti-AMV system, suggests the possibility that this plasma contained virus or viral antigens. This is in keeping with the work of Armstrong (12) who found two lines developing in immunodiffusion tests between AMV-infected chicken plasma and RSV hamster antiserum and that of Fleissner (13) who also showed two immunodiffusion lines between AMV viral antigens and "COFAL" serum.

Although viral antigens that react with various antisera have been described in cases of human leukemia (14), the present study is the first to report a cross-reaction of this

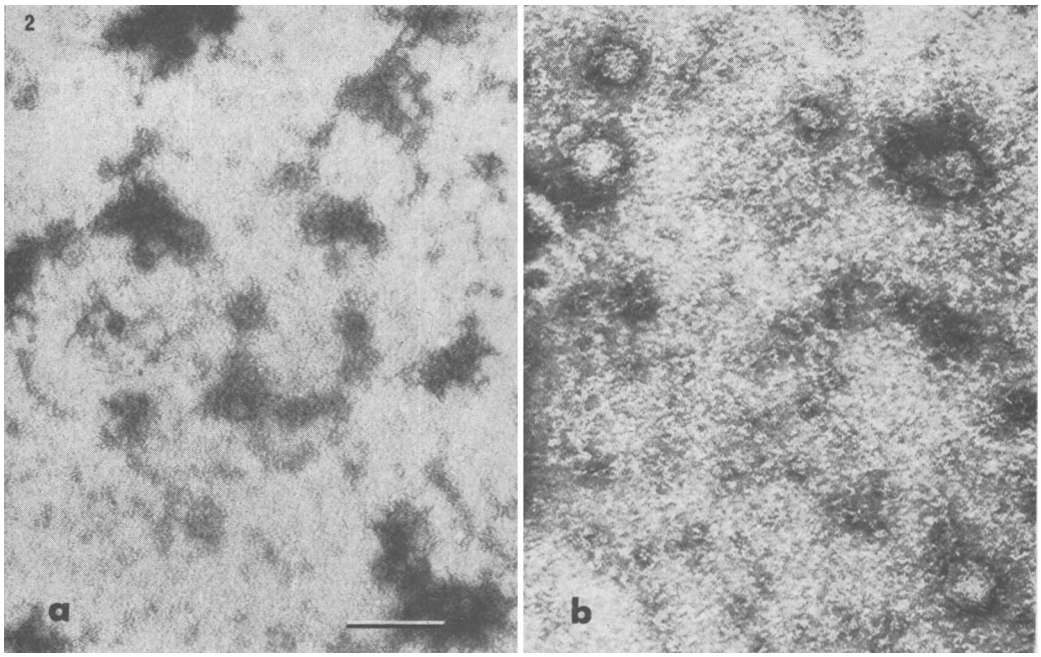

FIG. 2. Electron micrographs of (A) normal plasma exhibiting small 23-nm units in the plasma protein matrix, and (B) P.R. plasma containing virus-like particles 46–56 nm. Bar indicates 100 nm in electron micrograph.

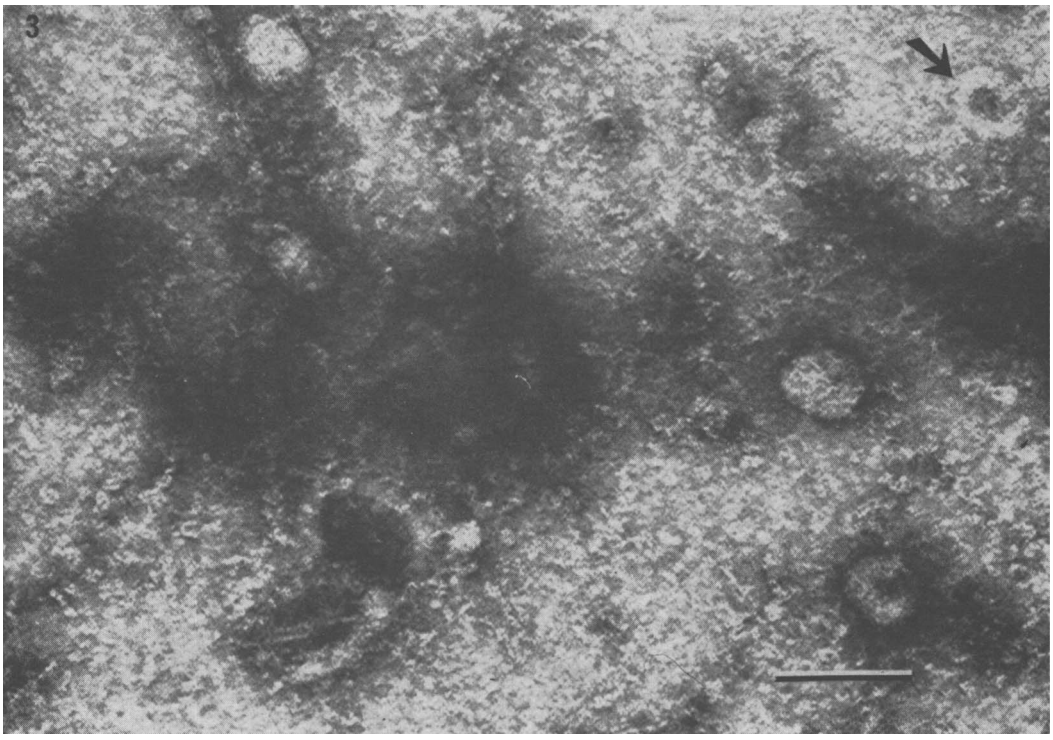

FIG. 3. P.R. plasma containing virus-like particles. One-fifth of these appear empty as indicated by arrow.

nature between antigens from a human leukemic source and antibodies directed against the avian leukosis GS antigens. Of concern was our inability to detect similar antigens in the plasma of the other leukemic patients studied. The problem may have been a quantitative one. The fulminating nature of P.R.'s leukemic state with a 24-hr doubling of peripheral blast cells, which is analagous to the leukemic state in AMV-infected chickens, could have caused an outpouring into the plasma of amounts of antigen detectable by the relatively insensitive immunodiffusion technique.

In view of the immunologic data and the presence of so-called virus-like particles in the electron micrographs, it is tempting to speculate that the antigen detected is, in fact, viral and related to the etiology of the leukemic state. However, even if one grants that the particles are indeed viruses, there is as yet no data to indicate that they are related either to the antigen detected or the disease.

Summary. The plasma of a patient with acute monomyelogenous leukemia when used as an antigen in Ouchterlony tests reacted with hamster serum containing anti-avian leukosis group-specific antibodies. Of the two immunoprecipitin bands seen, one merged in a reaction of identity with a band formed by AMV and the antibody-containing hamster serum. Since the leukemic plasma did not react with normal hamster serum, normal chicken plasma or anti-normal chicken serum in immunodiffusion tests, the results of this study represent a true cross reaction between antigens from a human leukemic source and antibodies directed against the avian leukosis group-specific antigens. Electron microscope studies on the human plasma revealed virus-like particles in a concentration greater than 5×10^7 particles/ml based upon direct counts. As yet the data do not allow for any correlations to be made between the immunologic findings and the presence of virus-like particles in the plasma.

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