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BRIEF COMMUNICATION

Depressed Antibody Response in the Mouse Infected with Rauscher Leukaemia Virus*

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Depressed antibody response has been described in human leukaemias and other reticuloses (Heath, Fairley and Malpas, 1964). Mice implanted with malignant cells or injected with Gross passage A leukaemia virus have also evidenced diminished circulating antibody levels and delayed rejection of skin homo- and heterografts (Fahey and Humphrey, 1962; Peterson, Hendrickson and Good, 1963; McCarthy, 1964; Dent, Peterson and Good, 1965). While possible causal relationships between immune stimuli and the development of leukaemia in man have been open to controversy, repeated injection of antigen has been reported to increase the incidence of reticular tumours in mice (Metcalf, 1962). The present study was directed toward exploring comparable relationships between antibody-producing and leukaemic systems in mice inoculated with Rauscher leukaemogenic virus (RLV) after immune stimulus with antigen in Freund's adjuvant.

The virus (Rauscher, 1962) was received in 1962 from Dr F. J. Rauscher, of the National Cancer Institute, Bethesda. Our sixth Balb/c mouse spleen passage of the virus was used here. Five- to 6-week-old Balb/c/jax female mice were given a single intraperitoneal injection of 0·25 ml of a complete Freund's adjuvant emulsion (Difco laboratories, Detroit) containing 1·25 mg bovine serum albumin (BSA) (Crystallized BSA from Pentex Inc., Kankakec, Ill., U.S.A.). Three days after immunization a group of ten mice were inoculated intraperitoneally with 0·20 ml of a supernatant of 10 per cent Rauscher virus-infected spleen homogenate clarified twice at 2500 rev/min for a total of 40 minutes in a PR-2 International refrigerated centrifuge. A second group of ten animals were inoculated similarly 10 days post-immunization. Mice were bled periodically by the orbital route and the sera assayed individually for antibody to BSA by the agglutination of fresh tanned, antigen-coated sheep erythrocytes. Total nucleated and differential cell counts were done on parallel blood samples, in order to follow the haematological development of the disease.

The depression of circulating antibody by infection with Rauscher virus is depicted in Fig. 1. As can be seen, the degree of antibody suppression was similar whether virus was given 3 days or 10 days after immunization. These mice, therefore, were treated as a single group of twenty animals for the enumeration of parallel haematological changes. Although at 22 days after inoculation of virus, eighteen of the twenty mice receiving virus after immunization showed depressed titres to BSA (Fig. 1), only one of the antibody depressed animals evidenced an elevated nucleated cell count (56,800 cells/mm^3). This count included increased numbers of lymphocytes as well as characteristic nucleated

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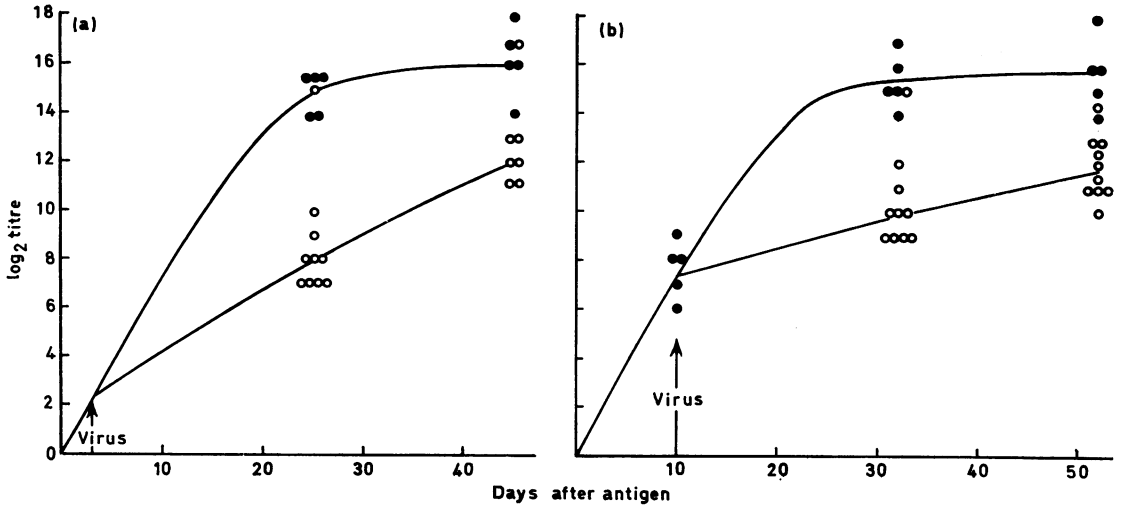

FIG. 1. Antibody response to BSA for immunized mice and immunized mice infected with Rauscher virus (a) 3 days or (b) 10 days after immunization. Each circle represents serum from a single animal. ●, Antigen only; ○, virus 3 days (a) or 10 days (b) after antigen.

erythrocyte components (Siegel, Weaver and Koler, 1964). The blood pictures of the other nineteen animals were normal, total white cell counts ranging from 3400 to 9950 cells (mean = 6115). This was comparable to the values obtained for the ten immunized-only animals (range 3700–7900, mean = 6165). After 42 days of infection, three of the immunized, virus-inoculated mice had died and thirteen showed definite haematological evidence of leukaemia, the mean nucleated cell count being 68,000 cells/mm^3, (range 17,260–283,200). At this time depression of antibody was still evident although the difference from control values was not as marked as seen at 22 days (Fig. 1).

The infectivity of Rauscher virus in mice previously immunized with adjuvant also appeared to be enhanced as compared to that in mice receiving virus alone. Sixty-three days after inoculation of virus, surviving mice were bled for haematology and were killed and spleens and thymuses were weighed. Infection was determined by the occurrence of splenomegaly with a peripheral blood leukaemia or by prior death resulting from virus infection. By these criteria, 5/10 of the mice receiving only virus showed infection compared with 18/20 of the adjuvant-immunized animals. It should be pointed out, in this connection, that the two mice in this latter group which failed to evidence leukaemic development were also the same two mice which failed to demonstrate depressed antibody titres (Fig. 1).

In an additional study, employing eight animals in each experimental group, virus was administered 18 days after immunization. When antibody titres before and 22 days after infection were compared, it was observed that levels of antibody had reached a plateau in the virus-infected mice, as compared with a rise in mean reciprocal titres from 4000 to 16,000 in the immunized controls. The infectivity in these mice pre-treated with adjuvant was also increased as compared with a control, virus-infected group. Seven out of eight of the former were found to be infected after 98 days as compared with 3/8 of the controls.

A possible mechanism whereby infection with Rauscher virus suppresses antibody is suggested by the present findings and by observations in this laboratory regarding the pathogenesis of the disease. This possible mechanism is a competition between virus and

antigen for a stem cell that has immunoproliferative potentials as well as being subject to neoplastic direction by the virus, these two courses of development being mutually exclusive. The primary target organ of Rauscher virus is the spleen and the accompanying leukaemia is characterized haematologically and histologically by an increase in a variety of abnormal and immature cell forms (Hopkins and Siegel, 1965). Prominent in the blood are nucleated cells of the erythrocytic series (Siegel *et al.*, 1964). There may also be present elevated numbers of lymphocytes, the appearance of megakaryocytes and, depending upon the functional state of the reticulo-endothelial system at the time of virus inoculation, granulocytic proliferation (Siegel, unpublished observations). No increase, however, in numbers of cells of the plasmocyte series has been observed. According to the stem cell theory of origin, these various series all derive from a common pluripotential form. The virus-infected stem cell, according to the present proposal, is no longer competent to become committed immunologically, although it can undergo proliferation and limited differentiation along the line of a number of other lymphoreticular cell types. In this regard Peterson *et al.* (1963), studying the suppression of phage neutralizing activity in mice infected neonatally with passage A virus, suggested possible viral interference with the role of the thymus in immunological maturation. In the case of the Rauscher virus, little or no thymic involvement has been observed grossly or microscopically (Siegel *et al.*, 1964) and mice thymectomized at 6 weeks of age and subsequently infected with RLV have shown neither depression nor enhancement of the disease (Siegel, unpublished observations). Also, in the present studies, mice were inoculated with virus at an age at which the role of the thymus in immunological mechanisms has been reported to be greatly diminished (Miller, Osoba and Dukor, 1965). These findings concerning the thymus would indicate the involvement of peripheral rather than central lymphoid tissues in the phenomena observed here.

Competitive interference at a stem cell level was also suggested by the finding that prior adjuvant stimulation rendered the mouse more receptive to infection. Out of a total of twenty-eight animals receiving antigen–adjuvant from 3 to 18 days before virus inoculation, twenty-five evidenced infection as compared with 8/18 of the mice receiving virus alone. It was previously suggested (Morton and Siegel, 1966) that one of the functions of complete Freund's adjuvant might be to stimulate the migration of peripheral small lymphocytes to fixed tissue sites preparatory to antibody formation, and Yoffey (1964) has presented evidence pointing to the small lymphocyte as a haemopoietic stem cell. Increasing the number of stem cells available to virus infection might therefore be expected to result in the observed potentiation of virus infectivity. Although RLV is in itself antigenic (Fink and Rauscher, 1964), its role as a leukaemia-inducing agent appeared in the present experiments to predominate over the effects of antigenic stimulation in determining the development of these pluripotential cells, whether the antigen was BSA or the virus itself. Fink and Rauscher (1964) observed that prior injection of either complete or incomplete Freund's adjuvant enhanced the resistance of mice to the Rauscher virus. These workers suggested that their Balb/c strain mice harboured in latent form this, or an antigenically similar virus and that the inoculation of Freund's adjuvant resulted in the production of neutralizing antibody. On the basis of the present findings, it would appear that the mice used in this laboratory either did not harbour such a latent virus, or that a competition of antigens engendered by the administration of BSA with the adjuvant made any immune response to latent viral antigen inadequate for protection against subsequent viral infection.

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