Serum Agglutinin Levels to Sheep Red Blood Cells in Mice Infected With Rauscher Virus.* (31516)

BENJAMIN V. SIEGEL AND JANE I. MORTON
Department of Pathology, University of Oregon Medical School, Portland

In a previous report from this laboratory (1) the depressed antibody response to bovine serum albumin (BSA) was described in young adult mice immunized with BSA in complete Freund's adjuvant 3, 10, or 18 days before infection with Rauscher leukemogenic virus. In these experiments antibody suppression was observed to occur even before hematological changes indicative of leukemia became evident. The present study was directed to defining further this antibody-depressing role of leukemia virus employing sheep erythrocytes as antigen. The results indicate that the degree of depression of the primary response is directly related to the extent of leukemogenesis at the time of immunization, while the secondary response seems to be influenced very little by this virus infection.

Materials and methods. The virus employed was obtained initially from Dr. F. J. Rauscher, National Cancer Institute, Bethesda, and had been through 7 serial mouse spleen passages in this laboratory. Female BALB/c mice, 6-7 weeks old, obtained from Jackson Laboratory, were inoculated intraperitoneally with 0.20 ml of 10^{-1} or 10^{-2} dilutions of Rauscher virus prepared as previously described(1). At selected times before or after virus administration animals were injected intraperitoneally with 0.25 ml of a 33% suspension of saline-washed sheep red cells.

Blood samples for antibody assay and for hematology were collected periodically from the retro-orbital plexus. These were diluted in the standard way for total nucleated cell counts. Differential determinations of Wright-Giemsa stained peripheral blood smears were performed by counting a field of 100 intact cells. Numbers of disintegrated nucleated cells present per field of 100 intact cells were also determined. Serum samples were stored at −20°C until completion of a particular study in order to carry out antibody assays of all samples at one time. Following inactivation at 56°C for 30 minutes, 2-fold serial dilutions of individual mouse sera were assayed in a micro-titer system. Mercaptoethanol-resistant antibody was determined by incubation of 1:6 diluted serum with 0.05 M 2-mercaptoethanol(2) for 30-60 minutes prior to titration.

Results. As shown in Fig. 1A, mice immunized with sheep cells on the same day as viral inoculation showed no significant changes in the primary antibody response at 4, 8, or 16 days after immunization. At 4 days most of the antibody was mercaptoethanol-sensitive and there was some indication of differences in the titers of mercaptoethanol-resistant antibody. Eight days or more following the primary immunization, and likewise during the secondary response, mercaptoethanol-sensitivity was no longer detectable. Antibody titers in infected mice were demonstrably less than the controls 4 days following the secondary stimulus and were lowest in animals receiving the 10^{-1} dose of virus. A retardation, in infected mice, of response followed by recovery to titers approaching those of the controls was indicated by the rapid increase in antibody levels between 4 and 7 days following the secondary stimulus. Results, not included here, with mice immunized 4 days following viral inoculation were almost identical to those immunized at zero time.

Fig. 1B delineates the results with mice immunized with sheep red blood cells 8 days following viral inoculation. As may be noted, the primary titers for infected animals were depressed relative to the controls. Also, serum antibody titers were lower in animals which had received the 10^{-1} dose of virus. The pattern of the secondary response was similar to that seen in mice inoculated with virus and immunized on the same day. There

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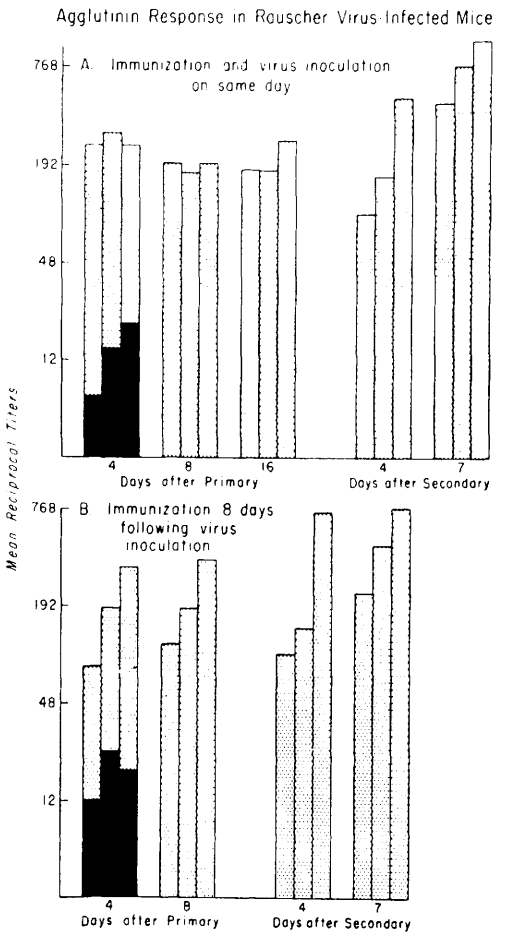

FIG. 1. Average agglutinin titers to sheep red blood cells in BALB/c mice at given times after primary and secondary immunizations. Antigen was injected on the same day as (A) or 8 days after (B) viral inoculation. Individual bars from left to right in each group represent 10^{-1}, 10^{-2} and 0 (control) viral inocula. Darkened areas represent corresponding mecraptoethanol-resistant antibody titers. Each bar represents average of 5 mice.

was an early delay in increase of antibody levels seen at 4 days, while by 7 days the magnitude of titer increases over the primary levels was similar for both virus-infected and control animals. In ancillary experiments, it was also observed that mice immunized 12 to 16 days after viral inoculation displayed depressed antibody titers similar to those seen with immunization after 8 days.

The extent of leukemogenesis in these virus-inoculated animals was determined by hematological changes including elevated nucleated cell counts, increased smudge cell numbers(3), and appearance in the blood of nucleated erythrocytes(4), V-cells(5) and atypical lymphocytes(3). The last 3 cell forms are frequently detected in the peripheral blood of Rauscher virus-infected mice before increased total cell counts are observed. On the 16th day following viral inoculation, mice which had received 10^{-1} virus and sheep red blood cells on the same day showed a slight increase in nucleated cell counts (mean = 14,610 cells/cu mm, range 10,150-17,350), and 2/5 mice showed V-cells and nucleated erythrocytes. Mice which had received sheep cells 8 days after 10^{-1} virus inoculation manifested a somewhat greater elevation in nucleated cell counts at 16 days, (mean = 25,540 cells/cu mm, range 22,400-29,350), 4/5 mice showed atypical lymphocytes and all had increased numbers of smudge cells in the peripheral blood. None of the 10 mice in the two 10^{-2} virus dose groups showed any hematological evidence of leukemia at this time. Total counts were normal (mean = 8,940 cells/cu mm, range 6,900-11,250), and no unusual cell types were observed.

In another study (Fig. 2), mice received a 10^{-1} dilution of virus on the 7th day following the second of two injections with SRBC given 24 hours apart. Primary antibody titers persisted at similar levels for mice in both the infected and control immunized

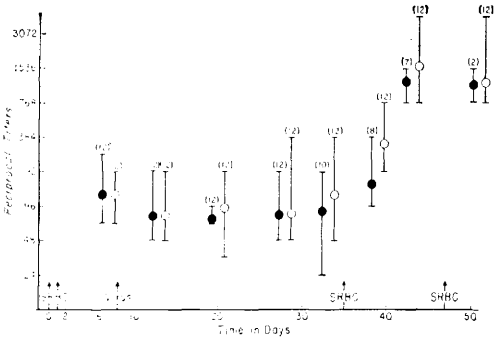

FIG. 2. Time course of production of agglutinins to sheep red blood cells (SRBC) in virus-infected and control mice. Ranges of reciprocal titers are shown by vertical bars with the respective means indicated for infected (●) and control (○) animals. Numbers of mice studied by titrations are shown in parentheses, and, in the case of infected mice, represent survivors. Days of SRBC injection and viral inoculation are denoted with arrows.

groups. A secondary stimulus was given on the 35th day. A slight lag in response in the leukemic animals was indicated by titers after 4 days, while levels comparable to the controls were seen by 8 days. Another SRBC injection on day 47 had no effect on circulating antibody levels as demonstrated by titers 4 days later. Most of the infected mice died between 30 and 50 days following the initial SRBC immunization (22-42 days post-viral inoculation), the period during which the secondary response was being studied. The peripheral blood picture at 33 days, just prior to administration of the secondary stimulus, indicated that all 10 surviving virus-inoculated animals were in advanced stages of leukemia. The mean nucleated cell count was 72,600 (range 24,500-177,000). Of these, approximately 30,000 were smudge cells and 24,000 were nucleated erythrocytes. At this time splenomegaly was prominent, hematocrits markedly reduced (25%) and the sera manifestly icteric. By way of comparison, control animals had nucleated counts of 8,800 cells per cu mm (range 6,400-13,600) with about 5% smudge cells and no abnormal cell types.

Discussion. The results described here for the immune response in Rauscher virus-infected mice are similar in a number of respects to the antibody situation observed with the human acute leukemias(6). In both instances, preexisting antibody levels and the secondary response are not depressed in the leukemic subject, while the primary response to new antigenic stimuli is usually diminished. In the present experiments, the almost complete lack of effect of a terminal leukemic state on the secondary immune response was in striking contrast to the depression of primary titers observed even before the appearance of leukemia development. These results, generally, support the hypothesis(1) that antibody suppression with Rauscher virus infection is due to a competition between antigenic information and infectious virus for a pluripotential stem cell, which has the capacity to respond to either one of these stimuli alone but not to both. The virus, according to this hypothesis, is without influence on the already committed immune cell, whether this cell is actively synthesizing antibody or merely contains within it the information for such synthesis. In this regard, it has been suggested(7) that activation of antibody formation in such latent, specifically committed cells may be responsible for the secondary response.

Depressed primary and secondary antibody responses have been observed recently with Friend virus(8), and neonatal infection of animals with murine lymphocytic leukemia viruses has been shown(9,10) to result in suppression of responses to subsequently administered antigen. Peterson *et al*(9) and Cremer *et al*(10) proposed interference with thymus-associated maturation of the peripheral lymphoid system as a possible mechanism for the suppressive effect. In the present experiments, only adult mice were used in a virus-induced leukemogenesis shown to be independent of thymic involvement(4,11).

Summary. Primary agglutinin titers were depressed in mice immunized with sheep red blood cells 8 days or more following inoculation of 10^{-1} or 10^{-2} dilutions of Rauscher leukemogenic virus. The extent of depression was proportional to virus dose and could frequently be detected before the appearance of hematological evidence of leukemic development. Immunization 7 days before, at the time of, or 4 days after, viral inoculation did not result in depression of primary titers. In all these instances a lag was noted in antibody response 4 days after the secondary stimulus; however, by 7 or 8 days increases in antibody titers over primary levels were similar in both virus and control groups. Even in mice markedly leukemic at the time of secondary challenge no depression of the secondary response was observed. A possible mechanism concerning the influence of viral leukemogenesis on the immune response is discussed.

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Healing of Urinary Bladder Wounds. Morphologic and Biochemical Studies.* (31517)

FINN RASMUSSEN (Introduced by G. Asboe-Hansen)

Connective Tissue Research Laboratories, Department of Dermatology, University of Copenhagen, Rigshospital, Copenhagen, Denmark

Connective tissue is ubiquitous and its elements play an important role in almost all physiologic and vital processes. Changes in the connective-tissue cells, ground substance and fibers predominate in wound healing. The cells produce acid mucopolysaccharides, and the fibrous tissue is important in restoration of structural continuity and production of adequate tensile strength. The regeneration process has been studied intensely, and parallelism between tensile strength, microscopic appearance of collagen, and hydroxyproline content has been established(1). Hydroxyproline is an imino acid specific of collagen. All repair processes are characterized primarily by edema, and secondarily by mucinous and fibrous organization of the water(2).

The purpose of this investigation was to study the morphologic changes occurring during healing of linear wounds in the urinary bladder, and to compare these observations with the biochemical findings in the same wounds.

Material and methods. Eighty-nine albino rabbits weighing about 2.5 kg were kept on an adequate laboratory diet for at least one week before operation. Seventeen of the animals served as controls. The animals were anesthetized by intravenous injection of 60 mg Nembutal® supplied with ether inhalation. The abdomen was opened through a low midline incision. The urinary bladder was emptied by puncture and aspiration. An incision through all layers was made in the midline of the anterior wall from the top to the neck. The bladder was closed in 2 layers with continuous sutures of 4-0 non-traumatic silk. In the first layer the suture was passed through the whole bladder near the wound edge. The second suture was seroserous. The abdominal wall was closed in layers with silk sutures. The animals were divided in 12 groups sacrificed 1, 2, 3, 4, 5, 6, 7, 9, 11, 14, 21 and 28 days, respectively, after the operation. They were killed by intravenous injection of 300 mg nembutal. The wounds were removed and specimens from the central part taken for histologic examination, while the rest was used for biochemical analysis.

Fixation and staining. The specimens were fixed in a fresh 4% aqueous solution of lead subacetate for 24 hours. This precipitating medium has been found to be the best for metachromatic staining of acid mucopolysaccharides(3,4). After embedding in paraffin, 7 μ thick sections were cut and stained with an 0.5% aqueous solution of toluidine blue, which stains acid mucopolysaccharides metachromatic. Parallel staining with haematoxylin-eosin, van Gieson-Hansen's connective-tissue stain for collagen, and orcein for elastic tissue was performed.

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