Retardation of Murine Viral Leukemogenesis by Prolonged Antigenic Stimulation

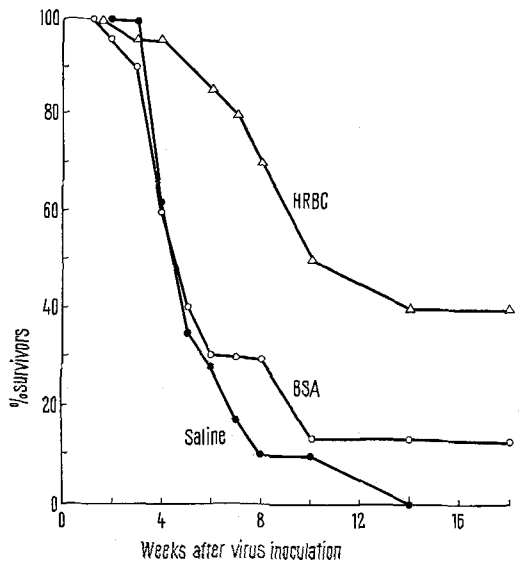
Infection of mice with RAUSCHER leukemogenic virus[1] has been demonstrated to have a markedly immunodepressive effect[2-4]. Studies from this and other laboratories[2,3,5] have suggested that this might be the consequence of competition between virus and antigen for a common pluripotential target cell. The concept of a common target cell has also been supported by observations that leukemogenesis, as well as the immune response, could be potentiated by prior injection with complete Freund's adjuvant[2,6]. On the other hand, intensive treatment of mice with non-viral-related antigens over a 5 week period resulted in leukemic retardation by what seemed to be a competitive process[7]. Thus, it appeared that leukemogenesis could be accelerated or retarded depending upon the adjuvancy or immunogenicity of the administered stimulus. The present study was directed to investigating the effects of long-term weekly injections of bovine serum albumin (BSA) and human type-0 erythrocytes (HRBC) on RAUSCHER leukemogenesis in BALB/c mice, in which the virus was administered 8 months after the initiation of antigen treatment. The findings have indicated that BSA administration had little effect on leukemogenesis, while HRBC resulted in marked retardation of the leukemic process.

In a preliminary experiment, 2 groups of 20, 5-week-old female BALB/c mice received 6 evenly-spaced i.p. injections of 0.2 ml of 1:3 diluted, saline-washed sheep erythrocytes (SRBC) or of 0.2 ml saline alone over a 3 week period. Five days after the last injection, 0.2 ml of a 10% RAUSCHER virus preparation[8] (seventh passage in this laboratory) was inoculated i.p. After 12 weeks, 12/20 of the SRBC-treated mice were dead as compared to 13/20 of the saline-treated animals; and, after 6 months, 14/20 and 15/20 in the SRBC and saline groups, respectively, were dead. These results demonstrated that with relatively few injections of cellular antigens there was little effect on the survival of infected animals. A more prolonged immunization schedule was then undertaken, as described below.

Three groups of thirty 9-week-old female BALB/c mice were started on one of the regimens of weekly injections consisting of 0.25 ml volumes of (1) 1.25 mg BSA (Pentex, Fraction V) in saline, (2) saline-washed human type-0 blood cells as a 33% suspension in saline, or (3) 0.85% saline alone. Weekly injections of BSA and HRBC resulted in anaphylactic deaths in 9/30 of the BSA-treated and 10/30 of the HRBC-treated mice between the second and fourth months of the experiment, deaths not occurring thereafter. One of the saline-injected mice died early, presumably from faulty injection procedure. At the age of 10 months, 0.2 ml of a 10% suspension of RAUSCHER virus (passage 8) was inoculated i.p. into all groups. No further antigen was administered subsequent to virus inoculation.

In the Figure are depicted percent survivals for the 3 groups of mice following virus inoculation. Saline- and BSA-treated animals showed little difference in initial death rates. However by 14 weeks, when all 29 mice in the saline-injected, virus-inoculated group were dead, 3/21 BSA animals were still alive and survived for an additional 1–2 months. In mice that had previously received HRBC injections, deaths were markedly retarded, 8/20 animals surviving the initial 14 week period. Seven months after virus inoculation, 5 of these mice were alive and, upon sacrifice, 4 showed little or no evidence of leukemia as assessed by hematology and splenomegaly (Table). Thymuses were normal in size or involuted, and liver weights were normal. Smudge cells, previously demonstrated to be associated with RAUSCHER leukemogenesis[7,9], were increased only in mouse No. 1. Although this animal showed some spleen enlargement, its peripheral nucleated blood cell count was not notably elevated.

A definite retardation of leukemogenesis was observed in the mice receiving weekly injections of human red blood cells over an 8 months period prior to RAUSCHER virus inoculation. Similar treatment with bovine serum albumin did not result in such retardation. One explana-



Percent survivors with time following RAUSCHER virus infection of mice pre-treated over a period of 8 months with saline, bovine serum albumin (BSA) or human red blood cells (HRBC).

Nucleated cell counts, spleen, thymus and liver weights of human red blood cell-treated mice surviving 7 months post-virus inoculation

Mouse No.	Nucleated blood cells/mm³	Smudge cells/100 nucleated cells	Organ weights spleen (mg)	thymus (mg)	liver (g)
1	10,800	70	993	involuted	1.13
2	4,400	8	315	15	1.45
3	7,200	4	145	25	1.33
4	7,900	18	286	19	1.32
5	8,300	8	227	43	1.55

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tion might be the differences in antigen dose employed, since mice in the BSA group received 1.25 mg BSA weekly while the HRBC group received inocula of 0.8 ml packed red cells. In this connection, a number of possible mechanisms could be invoked to account for the protective effect of HRBC administration. For example, HRBC may have stimulated formation of antibodies cross reacting with RAUSCHER virus. Alternatively, a non-specific protective effect might be obtaining, such as stimulation of interferon production[10] by the red cells or by virus contaminating these cells. Delayed deaths may also have been the consequence of decreased availability of cells susceptible to virus infection due to their prior commitment along immunologic pathways[7].

METCALF[11], employing weekly injections of BSA and *Salmonella flagellar* antigen, reported an increased incidence of reticular tumors in C3H mice. Increased reticular tumors were also demonstrated in animals following transfer of spleen cells from parent to F1 hybrid recipients[12] and between strains of mice differing at the H-1 histocompatibility locus[13]. Possible mechanisms suggested have included activation of oncogenic virus and stimulation of immunocompetent cells to neoplastic proliferation. DAMESHEK[14] has proposed that certain forms of leukemia may be due to abnormalities in immunoproliferation. Such differences from those findings of the present study may be due to differences in the host cell types involved in the neoplastic responses. If, as speculated[2-5], the target for RAUSCHER virus infection is a stem cell, availability of such cells may be influenced by the systemic requirements for differentiated cells. On this basis, it would be proposed that the demand for differentiated cell types participating in the response to foreign red cell antigens was sufficiently great to cause a diminution in numbers of precursor cells available for viral infection. Further studies are required to clarify the mechanism of this retardative effect on viral leukemogenesis and to determine its relationship, for example, to antigen dose and structure[15].

Zusammenfassung. Die Mortalität an Leukämie bei BALB/c-Mäusen wurde durch wöchentliche i.p. Injektionen mit menschlichen Erythrozyten herabgesetzt, wenn sie während 8 Monaten vor der Impfung mit RAUSCHER-Leukämievirus vorbehandelt waren. 7 Monate nach der Virusinfektion zeigten 20% dieser Mäuse keine Zeichen von Leukämie. Kontrollversuche mit der gleichen Serie von Injektionen von Ochsenserumalbumin zeigten keinen Schutz.

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Blastomitogenic Agents in Leguminosae and Other Families

Except for the pokeweed mitogen of *Phytolacca americana*[1], plants which have been reported to contain substances capable of stimulating blastomitogenesis in lymphocyte cultures have been limited to the family Leguminosae[2-5]. It is known that a plasmacytoid cell type appears in pokeweed stimulated cultures[6] in addition to the blast cell produced by both phytohemagglutinin (*Phaseolus vulgaris*) and pokeweed[2,6]. In searching for a possible differential effect of blastomitogenic agents from different sources, upon refractory lymphocytes, we have screened seed extracts from a variety of plant families for blastomitogenic activity. In doing so we have discovered 4 such agents in the seeds of plants from the families Compositae, Ephedraceae, Clusiaceae and Solanaceae.

Lymphocyte cultures from several healthy donors were prepared by gelatin sedimentation of defibrinated blood as previously described[7]. Saline extracts of the seeds were prepared and filtered through Millipore filters (0.45 μ). A 1 or 2 ml suspension of lymphocytes (1×10^6 lymphocytes/ml) in TC-199 containing 100 U of penicillin-streptomycin mixture and 20% autologous serum was cultured in each tightly stoppered Bellco disposable glass tube. The seed extracts were added to the tubes in volumes of 0.01 ml and 0.1 ml. For each group of cultures an extract of red kidney beans (*P. vulgaris*), prepared in the same manner, was used as the reference mitogen. Tubes to which no extract was added served as controls. The cultures were sacrificed at 48 or 72 h and smears were prepared and stained with Wright's stain. 500–1000 cells were counted on smears from 72 h cultures which appeared to contain more blasts than the control cultures. Cells categorized as transformed were 'blasts', i.e. large cells with fine chromatin, single or multiple large nucleoli, and abundant, blue cytoplasm, often containing vacuoles. These cells could be distinguished from the macrophages that were present in some of the cultures. Lymphocytes with increased cytoplasm, but little or no nuclear change were not classified as transformed.

Ninety seed extracts, representing 38 families were tested for blastomitogenic activity[8]. Those extracts demonstrating significant blastomitogenic activity at 72 h are listed in the Table.

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