Leukemia Virus Suppression of Antibody-Forming Cells: Ultrastructure of Infected Spleens

Abstract. *Infection of adult BALB/c mice with Friend disease virus results in a leukemia-like disease characterized by erythropoietic changes and splenomegaly. A marked depression of formation of cellular and serum antibody occurs in infected animals. Electron-microscopic examination of the ultrastructure of spleen sections from infected mice with depressed immunity revealed that virus particles can be detected only in immature blastlike lymphoid cells and not in plasmocytes characteristic of the immune response in spleens of noninfected mice immunized with sheep erythrocytes.*

When a murine tumor virus, such as Friend or Rauscher disease virus, infects a susceptible animal, a number of cellular changes occur in the lymphoid tissue (*1, 2*). However, morphologic characteristics of the disease, as well as splenomegaly and erythropoietic changes, do not become apparent until several days after infection. Development of leukemogenic diseases in adult mice infected with murine viruses is associated with a marked decrease in immunologic competence for both cellular and serum antibodies (*3–5*). Thus, serum antibody responses to a variety of antigens, such as sheep erythrocytes, bovine serum albumin, or microbial antigens, are apparently depressed in rodents previously infected with tumor virus. Infection as late as the day of immunization (or 1 day before) has resulted in significant immunodepression (*5*).

The aforementioned studies suggest that the induced immunologic unresponsiveness may be directly related to initial events associated with the disease process and may not be an unrelated secondary characteristic of leukemogenesis. In other studies in which rodents were inoculated with leukemia viruses at birth, and in which there was a long lag period (usually months) before development of overt disease, prolonged depression of the immune response was also noted (*6*). In such instances, the virus may have exerted its influence on the thymus, an organ necessary for proper development of immunologic function. Immunodepression in mice infected as adults with leukemia virus seems to be unrelated to thymus involvement (*3–5*).

The fact that infection of mice with leukemia viruses at the same time as immunization, or very shortly before, still leads to inhibition of immunity suggests that the suppression may be related to a direct effect of the virus on antibody precursor or stem cells, and not to nonspecific suppression of antibody-synthesizing cells. Studies with single cell assays, by which individual antibody-forming cells have been found in immunized mice infected with tumor virus, indicated that the immunologic defect may be due to a deficiency in production of cells capable of secreting antibody, and not to decreased synthesis of antibody by normal numbers of cells (*3, 5, 7*).

If virus affects stem cells, there may be a difference in distribution of viral particles between antibody-forming cells and undifferentiated stem cells in antibody-producing tissues early in the disease process when immune competence is only partially depressed. Electron microscopic examination of lymphoid tissue from immunized mice infected with a leukemia virus is used to determine the presence or absence of virus in cell types related to antibody formation.

Susceptible BALB/c mice were infected with Friend disease virus (FDV) and later immunized with sheep erythrocytes (*5*). The number of antibody plaque-forming cells (PFC) appearing in the spleens of these mice after immunization was counted by the localized hemolytic plaque assay in agar gel (*8, 9*). In addition, spleen sections from selected mice were prepared for examination by electron microscopy (*10*).

Normal mice immunized intraperitoneally with a standard concentration of sheep erythrocytes [4×10^8 red blood cells (RBC) per mouse] had a vigorous immune response (Table 1). Large numbers of hemolysin-secreting cells appeared in the spleens of such animals; the peak was reached 4 days after immunization, and the number of cells decreased rapidly thereafter. On the other hand, mice infected with FDV on the same day as immunization had a markedly suppressed antibody plaque response; only about one-half the number of PFC appeared in their spleens, compared to that appearing in the spleens of uninfected controls.

There was only a slight depression of the serum titers to RBC in these mice, as compared to controls. When infection occurred 3 or 7 days before immunization, we detected a marked depression in the number of antibody-forming cells in the spleens of these animals in comparison with those of the controls. The antibody titer of the serum was also depressed. Infection 12 days or more before immunization generally resulted in almost complete suppression of the immune response of these mice, many succumbing to the disease by the 2nd week after immunization.

Histologic examination of spleens of normal and infected mice immunized with RBC indicated an alteration in the cellular morphology and architecture of spleens of the virus-treated mice

Table 1. Immune plaque response (per whole spleen) of mice (at least four to six per day per group) infected with Friend disease virus. Day of infection relative to day of immunization: A, 2 days after; B, same day; C, 3 days before; D, 7 days before; E, 12 days before. Each animal was injected intraperitoneally with 4×10^8 sheep erythrocytes.

Infection	Day after immunization						Mean peak of antibody titer ($\log_2$)
	0	2	4	7	10	15	
				Infected mice			
A	78	2659	61,408	5860	1140	640	8.1
B	89	1260	29,500	5386	1365	584	7.8
C	68	1051	7,340	985	415	395	6.9
D	80	378	1,255	655	538	360	5.8
E	75	157	165	248			3.9
				Noninfected mice			
	85	3550	68,900	9850	1465	765	8.4

compared to those of normal controls. Spleens of noninfected mice 4 days after immunization had many typical lymphoid follicles containing numerous germinal centers. Mice infected 3 or 7 days before immunization had less differentiated white and red pulp, with a striking absence of normal germinal centers.

Electron-microscopic examination indicated that the germinal center areas and the lymphoid follicles of the spleens from noninfected immune mice had large numbers of cells which appeared to be plasma cells and immature lymphocytes. Such cells had well-differentiated nuclei and a large amount of cytoplasm containing organized endoplasmic reticulum and ribosomes (Fig. 1a). The ultrastructure of these cells was similar in most respects to that of cells described by many investigators as antibody-forming cells in the center of hemolytic plaques in agar gel; such cells were absent in spleens of infected mice.

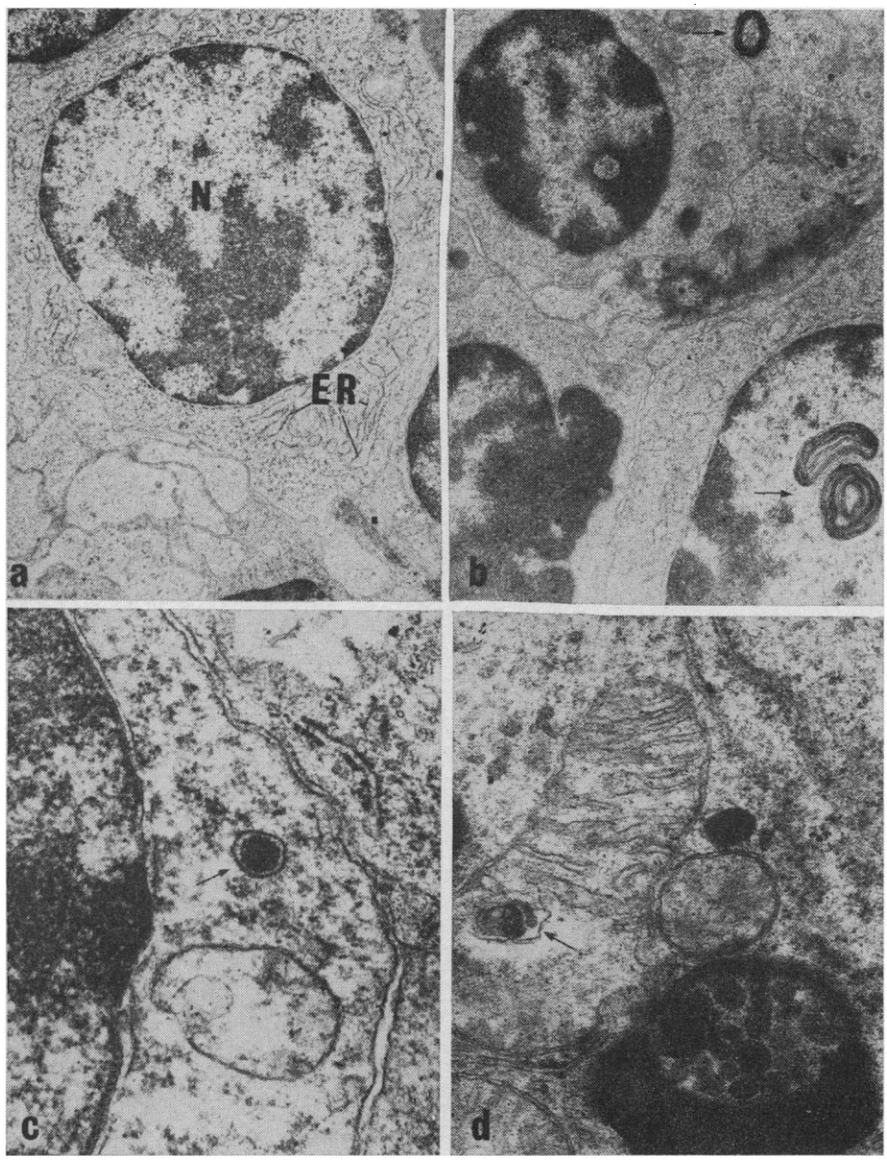

Fig. 1. Ultrastructure of spleen sections from mice immunized intraperitoneally with 4×10^8 sheep erythrocytes 4 days previously. Noninfected mice had an average of 60,000 or more PFC per spleen (150 to 200 PFC/10^6 leukocytes), whereas mice infected 7 days prior to immunization had less than 2000 PFC per spleen (1 to 5 PFC/10^6 leukocytes). (a) Typical plasmocyte from normal mouse after immunization, showing widened channels of endoplasmic reticulum (*ER*) and condensed chromatin (× 10,000). *N*, nucleus. (b) Parts of three lymphocytic blast cells from spleen of mouse injected with FDV (dosage, 10^{-1}) 7 days prior to immunization. Two cells show unique structures with concentric membranes (arrows) peculiar to infected mice (× 10,000). (c) Part of cytoplasm and nucleus of a blast cell from spleen of mouse injected with FDV 7 days before immunization. Single virus particle (arrow) is present, with electron-dense nucleoid, surrounded by an outer double membrane (× 52,000). (d) Part of cytoplasm of a blast cell from spleen of mouse 11 days after injection with FDV (dosage, 10^{-1}) and 4 days after immunization with sheep erythrocytes. A viral inclusion body is in the lower right. A virus-like particle is seen in the mitochondrion (arrow) (× 52,000).

Spleens from animals injected with a 10^{-1} dilution (approximately 100 median lethal doses) of a stock preparation of FDV 7 days before immunization and sacrificed the 4th day after immunization, had mainly large undifferentiated cells in the white pulp. Figure 1b shows part of a typical large cell similar to undifferentiated blast cells or plasmoblasts. Most of these cells had ribosome clusters and some endoplasmic reticulum, but they were morphologically distinct from the usual cell type in spleens of noninfected mice. Virus particles were observed in most of these blastlike cells, often either at the cellular membrane or in clusters within the cytoplasm (Fig. 1, c and d). In some instances, there were marked alterations in the nuclei of these cells (Fig. 1b), as well as increased mitosis. Spleens of mice infected on the day of immunization had fewer morphologic differences than those of the controls. There were a few recognizable plasma cells and typical lymphocytes. However, no virus particles could be found in such cells; they were observed only in large undifferentiated cells. Several thousand cells in many thin sections from about 12 mice infected with virus were examined by electron microscopy. Typical plasmocytes were rare, and less than two per 1000 blast cells were detected in most spleen sections. Examination of over 48 cells with morphologic characteristics of mature plasma cells did not reveal any virus particles. Numerous adjacent cells morphologically similar to blast cells contained recognizable virus particles.

When the concentration of virus inoculum was varied from undiluted to 10^{-2}, there was a gradation in suppression of the immune response, as well as in morphologic changes. However, in all infected mice there was an increased number of undifferentiated nucleated splenic cells, most with visible virus particles. The virus particles were not found in cells which appeared to

be plasmocytes or mature lymphocytes.

Thus, the marked immunodepressive effect of a murine leukemia virus such as FDV may be related directly to morphologic alterations in the spleen, a tissue in which more than 95 percent of the antibody-forming cells appear (in normal mice) after a single immunization with sheep erythrocytes (*9*). Whereas noninfected mice immunized with RBC have a rapid development of PFC, as well as a marked increase in germinal centers and characteristic morphologic changes, mice infected with FDV do not have a similar cellular response. However, even infected mice produced some antibody—generally less than 5 to 10 percent of the PFC observed in normal controls. Electron-microscopic examination of sections of spleens from these animals revealed mainly undifferentiated large cells, most with virus particles. There was no evidence that similar virus particles were present in cells which, according to morphologic characteristics, could be engaged in antibody synthesis. Studies on the ultrastructure of single cells in the center of hemolytic plaques, obtained by plating dispersed spleen-cell suspensions from normal and FDV-infected mice immunized with sheep erythrocytes, should reveal whether or not virus particles can be detected in demonstrable antibody-producing cells.

Our observations and previous studies on the kinetics of antibody formation in animals infected with tumorigenic viruses suggest that the immunologic defect caused by such a virus could be in the stem cell stage. Other studies of transfer of spleen cells from virus-infected mice into immunologically incompetent recipients have indicated a marked deficiency in the number of "antigen-sensitive target cells" (presumably stem cells) found in large numbers in spleens of normal, noninfected donor mice (*7*). Thus, the leukemia virus may compete with antigen for specific stem cells. This does not seem to be merely an antigenic competition because there is no evidence that virus infection, either with or without immunization with red cells, results in stimulation of cells that appear to be morphologically similar to antibody-forming cells. Apparently, infection with the leukemia virus that we used results in a marked decrease in appearance of such cells.

Histologic examination indicates that the marked hyperplasia of spleen cells, with appearance of typical tumor cells, does not occur until 1 week or more after infection with FDV (*2*). However, electron-microscopic examinations indicated that most lymphocytes found in the red and white pulp of mice infected 1 week previously with virus and then immunized with sheep erythrocytes appear to be undifferentiated large nucleated cells without the morphologic characteristics of antibody-forming cells. Whether similar morphologic changes may be correlated with immunodepressive effects of leukemogenic viruses other than FDV is unknown.

If a virus infection alters the ability of lymphoid cells to respond to antigens during the stage prior to clinical symptoms of leukemia, there might be, for such a tumor virus, a selective advantage in which the host animal might not respond immunologically to the viral agent or to any other new antigen that may be associated with the developing neoplasia. Viral tumorigenesis results in appearance of new antigens (*11*). If such is the case with a leukemia virus such as FDV, a concomitant, generalized suppression of immune responsiveness may aid survival of new tumor cells in the host.

GLORIA CHAN, MARTHA W. RANCOURT
WALTER S. CEGLOWSKI
HERMAN FRIEDMAN
Departments of Microbiology, Temple University School of Medicine, and *Albert Einstein Medical Center, Philadelphia, Pennsylvania*

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Infrared Receptors in the Facial Pits of the Australian Python Morelia spilotes

Abstract. *There is a series of pits in the scales of the rostrum and posterior portion of the lower lips in some pythons and boas. In the Australian python* Morelia spilotes, *these pits are innervated by the maxillary and mandibular branches of the trigeminal nerve. Structural and neurophysiological evidence indicate that in the pits there are receptors that function as detectors of radiant heat flux.*

In pythons and boas, the rostral scales, anterior upper labial scales, and posterior lower labial scales often have depressed centers which, if sufficiently deep, may be referred to as pits (Fig. 1). These pits vary in number and depth in different species and are even absent from some species. Noble and Schmidt (*1*) have compared the pits of pythons and boas in structure and function to the facial pits of pit vipers (Crotalidae). The histological structure of the crotalid pits has been examined with light microscopy (*2*) and with electron microscopy (*3*). Bullock and Diecke (*4*) reported on the function of the crotalid facial pit as an infrared receptor. Not much information is available about the rostral and labial pits of pythons.

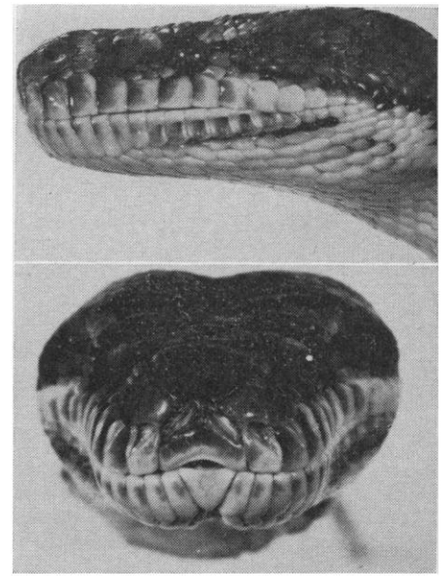

Fig. 1. Facial pits on the lower lip (upper) and rostrum (lower) in the Australian carpet snake *Morelia spilotes*. Pits on the rostrum have high posterior margins which cause them to be directed forward; those on the lower lips are directed laterally and only slightly forward.