histamine to anesthetized dogs while measuring arterial and gastric venous histamine concentrations. When histamine was infused at the low rates of 0.015 or 0.02 mg base per kg per hr, acid secretion began before there was a detectable (less than 1 $\mu$g per liter) rise in gastric venous histamine concentration.

*Summary.* The effect of ethanol upon the barrier function of the stomach was studied by irrigating pouches of the canine oxyntic glandular mucosa with solutions of ethanol ranging from 0.5 to 27% w/v. Solutions of 8% or less did not damage the mucosal barrier as judged by the fluxes of $Na^+$, $K^+$ and $H^+$. Solutions of 14 and 27% broke the barrier, for fluxes of the ions across the mucosa were greatly increased during and following ethanol application. In contrast to acetylsalicylic acid which is much more damaging in acid than in neutral solution, ethanol has essentially the same effect upon the barrier when applied in 100 mM HCl or in 30 mM phosphate buffer, pH 7.5. The rate of absorption of ethanol is a linear function of its concentration. The damaging effect of ethanol depends upon the concentration in contact with the mucosa and not upon the quantity absorbed. Low concentrations of ethanol (8% or less) stimulate acid secretion. Histamine could not be detected in the arterial or gastric venous blood of anesthetized dogs when the stomach was filled with 8 or 14% ethanol.

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## Suppression of the Primary Antibody Plaque Response of Mice Following Infection with Friend Disease Virus.* (32533)

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A number of murine viruses may induce leukemia-like diseases upon injection into susceptible strains of mice(1). Several of these viruses apparently induce neoplasia only upon administration to immunologically incompetent animals, such as neonates or surgically thymectomized mice(2). On the other hand, other tumorigenic viruses, such as Rauscher Disease Virus (RDV) and Friend Disease Virus (FDV), result in rapid development of splenomegaly and leukemia-like disease when

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inoculated into adult mice(3,4). These leukemogenic viruses are believed to affect primarily cells of the lymphocytic series.

It seems possible that the "target" cell for such virus infection may be a "lymphoblast" which, under normal conditions, may differentiate into a cell of the plasmocytic series following antigenic stimulus and secrete detectable levels of specific antibody. Infection of an animal with a leukemogenic virus, such as RDV or FDV, may result in early transformation of lymphoblast cells into a malignant state so that these cells, or their progeny, are no longer competent to respond to an antigenic stimulation. Such possible transformation of immature lymphoid cells to a neoplastic state, under the influence of a tumorigenic virus, may be an important mechanism whereby an individual adult animal succumbs to the virus infection, possibly without a demonstrable immunologic response to the virus or the developing neoplasm.

There have been a number of recent reports that circulating antibody levels in mice to a variety of antigens may be markedly reduced following induction of leukemia-like disease (5-9). There have also been studies indicating that lymphocytic cells isolated from humans with chronic lymphocytic leukemia exhibit immunological incompetence(10). The purpose of the present study was to investigate the temporal relationship between antibody formation, on the cellular level, with development of leukemia-like disease in mice following infection with a known leukemogenic virus. Cellular antibody formation was studied by means of the hemolytic plaque technique in agar gel(11,12). The effect of infection with a tumor virus on immunologic competence was determined by comparing the number of antibody plaque forming lymphoid cells in spleens and lymph nodes of normal mice with those of experimental mice infected with Friend Disease Virus prior to or following an immunizing injection with sheep erythrocytes.

*Methods and materials. Virus preparation.* Friend Disease Virus was obtained from the American Type Culture Collection as a lyophilized homogenate of infected murine spleen tissue. This preparation was passaged in Balb/c mice as a clarified 10 percent spleen cell homogenate at 21 to 28 day intervals.

*Experimental animals.* Inbred male Balb/c mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. The mice were usually 5 to 6 weeks old at the start of each experiment. The animals were maintained in plastic cages in groups of 6 to 10, and fed standard laboratory mouse pellets and water *ad libitum.*

*Antigen.* Sheep red blood cells (S-RBC), obtained commercially, were washed 3 times in physiological saline in the cold by centrifugation and resuspended in saline to a photometrically determined standard concentration of 20 percent v/v ($4 \times 10^8$ RBCs per ml).

*Immunization.* Mice were immunized by intraperitoneal (i.p.) inoculation of 0.5 ml of the washed sheep red cell suspension.

*Infection procedure.* Mice were infected by i.p. inoculation of 0.1 to 0.5 ml of the clarified stock spleen cell homogenate. Splenomegaly was used as the indicator of infection. Histological examinations were made of selected spleens and other lymphoid organs of specific mice at autopsy.

*Localized hemolytic plaque technique in agar gel.* The antibody cell plaque assay in agar was performed as described previously (12). In brief, 0.1 ml of a freshly prepared 10% spleen cell suspension from experimental and control mice was rapidly mixed with 2.0 ml melted 0.7% Noble Agar containing dextran and washed sheep red blood cells. All cell suspensions were tested in triplicate at several cell concentrations, usually $2 \times 10^5$ to $5 \times 10^6$ nucleated lymphoid cells per test plate. The plates were incubated at 37°C for one hour and then treated with guinea pig complement, diluted 1:20, for an additional 30 minutes at 37°C. The resulting zones of hemolytic plaques were regarded as being due to high efficiency 19S hemolysin formation secreted by individual lymphoid cells(12). In those experiments in which the numbers of antibody forming cells were enumerated late in the primary immune response, the number of low efficiency 7S antibody forming cells was enumerated by an indirect enhancement technique, in which additional sets of plates were incubated as above followed by treatment with a dilute preparation of rabbit anti-mouse gamma globulin and complement(13).

This additional treatment with the anti-globulin serum resulted in appearance of new plaques, regarded as due to low efficiency 7S hemolytic antibody to the sheep erythrocytes (13). The number of 7S plaques was estimated by subtracting the number of plaques counted on plates prior to use of anti-globulin serum from the number observed after enhancement(13).

*Results.* The cellular antibody response to sheep red blood cells was tested in mice infected at various time intervals with virus, and compared to the response observed in control mice immunized at the same time with red cells only. For these experiments, groups of 30 or 40 mice were injected with Friend Disease Virus at various time intervals prior to immunization with sheep erythrocytes. At various times after injection of the red cells, 4 or more mice were sacrificed from each group, spleen cell suspensions were prepared, and the number of 19S and 7S hemolysin-forming cells enumerated. Control mice, not infected with virus, exhibited a typical primary type cellular immune response during the first few days after immunization with the red cells (Fig. 1). There was a rapid increase in the number of hemolysin forming cells over the "background" count of 10 to 50 plaques per spleen. The peak number of hemolysin-forming cells was detected four days after immunization, with a gradual decline thereafter.

Mice injected simultaneously with both FDV and S-RBC had a slight but definitive depression of the early cellular immune response (Fig. 1). The most consistent difference was a more rapid decrease in 19S plaque forming cells late in the immune response.

As the interval between injection of virus and RBC immunization increased, the degree of depression of the number of antibody forming cells detectable in the spleens also increased (Fig. 1). Mice infected 21 days prior to immunization exhibited a profoundly depressed ability to respond to the sheep red

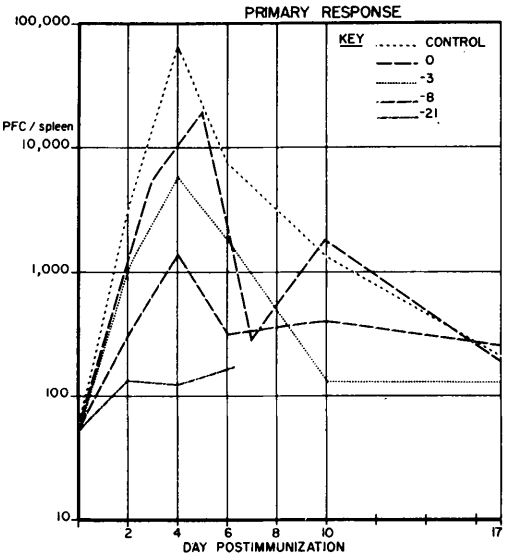


FIG. 1. Number of antibody plaque forming cells in spleens of control and Friend Disease Virus infected adult mice following primary injection with $4 \times 10^8$ sheep erythrocytes. Each curve represents average of at least 4 to 6 mice sacrificed at 2 to 3 day intervals. Numbers in key indicate time interval, in days, between virus injection and immunization.

TABLE I. The Peak Cellular Antibody Response of Young Adult Mice Injected 4 Days Previously with $4 \times 10^8$ Sheep RBC and Either Uninfected, or Infected at Various Times with Friend Disease Virus. At least 6 mice per group.

| Mouse group | Avg spleen wt (mg) | No. of plaques per spleen | % of control | No. of plaques per $10^6$ cells | % of control |
|---|---|---|---|---|---|
| Control | 165 | $6.82 \times 10^4$ | — | $4.2 \times 10^2$ | — |
| Infected 2 days after immunization | 176 | $6.20 \times 10^4$ | 91 | $1.9 \times 10^2$ | 45 |
| Infected immediately prior to immunization | 520 | $1.90 \times 10^4$ | 28 | $2.7 \times 10^1$ | 6 |
| Infected 3 days prior to immunization | 661 | $5.75 \times 10^3$ | 9 | $9.3 \times 10^0$ | 2 |
| Infected 8 days prior to immunization | 846 | $1.25 \times 10^3$ | 2 | $1.5 \times 10^0$ | .4 |
| Infected 21 days prior to immunization | 2640 | $1.24 \times 10^2$ | .2 | $1.9 \times 10^{-2}$ | .005 |

cells. Although there was a marked splenomegaly at this time, many mice had a minimal immune response during the first week after immunization. By 17 days after immunization, the number of antibody-forming cells of control and infected mice appeared to be within a rather narrow range of values, approximately four times that of the background level of unimmunized mice.

Table I summarizes data concerning the peak number of antibody-forming cells in spleens of control and FDV infected mice, as well as total spleen weights of these animals at the time of sacrifice. The spleen size increased markedly with increasing time interval after infection. Injection of mice with FDV 2 days *after* immunization had little or only slight effect on the total number of detectable hemolysin forming cells in the spleens at the time of peak antibody response. Infection of mice on the same day as immunization resulted in a slight to moderate, but consistent, reduction in the number of plaque forming cells per spleen. This depressive effect was most striking when the number of plaque-forming cells per million nucleated leukocytes was compared between normal control and infected mice. There was a 94% decrease in the average peak plaque response, when enumerated as the number of plaques per $10^6$ leukocytes plated, even when mice were infected on the day of immunization. As the interval between infection and immunization increased, the number of antibody forming cells in control and infected mice 4 days after immunization continued to differ markedly.

Late in the primary response spleens from normal mice formed many hemolytic plaques regarded as being due to 7S antibody (Table II). Usually over 70% of the total number of plaque forming cells of normal mice 17 days after immunization could be detected only by enhancement with anti-gamma globulin and were considered to be due to 7S antibody. Mice infected 2 days after immunization formed only slightly fewer enhanceable plaques (7S) when tested 17 days after administration of red cell antigen. Mice infected simultaneously or prior to immunization had very few additional plaques detected by the enhancement technique. The depression in the number of low efficiency 7S antibody producing cells late in the primary response was most marked in those animals infected with virus 3 days or more prior to immunization.

*Discussion.* The present study has presented information that infection of mice with Friend Disease Virus prior to or simultaneous with immunization with sheep red blood cell antigens results in a marked depression of the number of antibody forming cells as determined by the localized hemolytic plaque procedure in agar gel. The mechanism whereby such virus infection results in immune depression is unknown. One possible interpretation may be that FDV has a direct effect on antibody synthesizing cells, presumably lymphocytes or plasma cells, similar to the report of Medzon and Vas who observed a depression of secretion of humoral antibody when spleen cell suspensions were treated *in vitro* with Newcastle Disease Virus(14).

The depressive effect could conceivably be due also to possible competition between the leukomogenic virus and the red blood cell antigen for precursor cells, which otherwise may be directed into a sequence of events leading to antibody production. Prior contact of such cells with FDV presumably could result in a "misdirection" of the cell by virus into a neoplastic state and a concomitant in-

TABLE II. The Number of High Efficiency (19S) and Low Efficiency (7S) Antibody Plaques in Spleens of Mice 17 Days After Immunization with $4 \times 10^8$ Sheep Erythrocytes and Infected with Friend Disease Virus at Various Time Intervals. At least 6 mice per group.

| Mouse group | Plaques per spleen | |
|---|---|---|
| | Direct (19S) | Enhanced (19S + 7S) |
| Control | $2.07 \times 10^2$ | $3.98 \times 10^3$ |
| Infected 2 days after immunization | $1.78 \times 10^2$ | $1.77 \times 10^3$ |
| Infected just prior to immunization | $1.97 \times 10^2$ | $3.24 \times 10^2$ |
| Infected 3 days prior to immunization | $1.70 \times 10^2$ | $2.41 \times 10^2$ |
| Infected 8 days prior to immunization | $1.55 \times 10^2$ | $2.02 \times 10^2$ |

ability to form antibody. The results of the present study lends some support to this suggestion. Infection prior to immunization had the most profound depressive effect upon the cellular immune response. Infection after immunization had less effect on the same type of immune response.

It is not known at present whether a single antibody-forming cell may synthesize simultaneously or sequentially both 19S and 7S antibody. The effect of FDV infection of mice on appearance of low efficiency 7S producing cells late in the immune response following a primary immunization with red cells cannot be attributed with certainty to a direct effect of the virus on either precursor or active antibody synthesizing cells. However, it seems probable that suppression of appearance of 19S hemolysin forming cells may be directly related to the suppressed appearance of 7S hemolysin secreting cells. Virus infection may interfere with the normal sequence of conversion of one cell type to another, or in the number of precursor cells which are capable of being stimulated to form 7S antibody.

It seems unlikely that there is a shift of formation of antibody from the spleen to other organs, since other studies, not reported here, have shown that appearance of antibody plaque forming cells in peripheral and mesenteric lymph nodes are also markedly depressed at the same time that there is a marked reduction in the number of splenic plaque forming cells. Likewise, hemolysin titers were markedly depressed in these mice.

Several other studies with mice infected with leukemogenic viruses, including Rauscher Leukemia Virus, Moloney Leukemia Virus, Gross Passage A Virus, as well as Friend Disease Virus, have also indicated that there may be a marked depression of circulating antibody responses at various times following virus infection and immunization with antigens such as sheep erythrocytes(6,7,9), bovine serum albumin(8), or $T_2$ bacteriophage(5). The results of these studies indicate that the leukemogenic effects of viruses are associated with profound changes in cellular antibody formation by lymphoid cells. Further studies concerned with the mechanism of this relationship, including cell transfer studies, are in progress.

*Summary.* Infection of mice with Friend Disease Virus prior to or simultaneous with immunization with sheep red blood cells resulted in a marked suppression of appearance of antibody forming cells, as determined by a localized hemolytic plaque technique in agar gel. Infection with virus had a marked suppressive effect on the number of 19S hemolysin forming cells in mouse spleen tissue at the peak of the expected immune response and during a period of two weeks or longer thereafter. Appearance of low efficiency 7S hemolysin forming cells late in the immune response, following a single injection of sheep red cells, was also depressed in infected mice. Animals infected 3 days to a week or longer prior to immunization had the lowest number of detectable 19S or 7S antibody-forming cells. The number of plaque forming cells per million leukocytes tested was most suppressed in those mice which had obvious splenomegaly due to virus infection.

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