Conclusion. The $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins of mice differ in their electrophoretic migration. The $7S\gamma_{2a}$ globulin was seen to be slower electrophoretically than $7S\gamma_{2b}$ globulin in the four inbred strains of mice examined and $7S\gamma_{2a}$ globulin free of $7S\gamma_{2b}$ globulin could be obtained by Pevikon-block electrophoresis. Antiserums prepared in rabbits and sheep by inoculation with whole mouse serum frequently contain antibody specific for the $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins as well as the $7S\gamma_1$ globulin, and these specificities can be detected on routine immunoelectrophoresis.

1. Fahey, J. L., Wunderlich, J., and Mishell, R., J. Exptl. Med., **120**, 223 (1964).
2. Fahey, J. L., Wunderlich, J., and Mishell, R., J. Exptl. Med. **120**, 243 (1964).
3. Kelus, A. and Moor-Jankowski, J. J., Nature, **191**, 1405 (1961).
4. Herzenberg, L. A., Warner, N. L., and Herzenberg, L. A., J. Exptl. Med., **121**, 415 (1965).
5. Lieberman, R., Dray, S., and Potter, M., Science, **148**, 640 (1965).
6. Warner, N. L., Herzenberg, L. A., and Goldstein, G., J. Exptl. Med., **123**, 707 (1966).
7. Ovary, Z., Barth, W. F., and Fahey, J. L., J. Immunol., **94**, 410 (1965).
8. Coe, J. E., and Feldman, J. D., Immunology, **10**, 127 (1966).
9. Coe, J. E., J. Immunol., **96**, 744 (1966).
10. Muller-Eberhard, H. J., Scand. J. Clin. Lab. Invest., **12**, 33 (1960).
11. Coe, J. E., Immunochemistry, **3**, 427 (1966).
12. Ovary, Z., Bloch, K. J., and Benacerraf, B., Proc. Soc. Exptl. Biol. Med., **116**, 840 (1964).
13. Coe, J. E., Science, **155**, 562 (1967).

Received August 28, 1967. P.S.E.B.M., 1968, Vol. 127.

Effects of Antilymphocyte Serum on Virus Oncogenesis (32657)

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It is generally accepted that virus-induced tumors have antigens demonstrable by transplantation resistance or other methods (1,2). Much remains to be learned however, about the role of immune reactions in controlling tumor growth. Experiments with immunosuppressive procedures are helpful in the analysis of this problem. In general, chemical immunosuppressive agents have limited application because they may also affect virus or tumor cell multiplication, and this can make interpretation of results difficult. Comparisons of tumor incidence in neonatally thymectomized and intact animals exposed to viruses or chemical carcinogens have already provided much useful information (3–5). Mice or rats infected as newborns or later with polyoma virus, simian virus 40 or adenovirus type 12 consistently show more tumors after neonatal thymectomy than intact animals infected at the same time. Animals treated with chemical carcinogens sometimes show increased incidence of tumors after neonatal thymectomy, but usually do not. Mice infected as newborns with the mammary tumor agent or Passage A (Gross) or MLV (Moloney) leukemogenic viruses develop fewer mammary tumors or leukemias after neonatal thymectomy than do intact animals infected with these agents (3, 6–8).

Inhibition of leukemogenesis cannot be attributed merely to the removal of thymic cells sensitive to neoplastic conversion. Law and Potter (9) and Carnes *et al.* (10) found that in thymectomized animals restored by genetically identifiable thymus grafts the leukemias which developed were often of host origin and did not originate from the cells or donor thymic tissue. These findings suggest that nonlymphoid elements of the thymus (epithelial and reticular cells) facilitate the conversion of normal to leukemic cells within the thymus. Nevertheless the effect of thymectomy is clearly something other than immunosuppression, and analysis of the role of immunity in virus leukemogenesis calls for the application of a different immunosuppressive procedure.

Interest has been aroused recently by the use of antisera against lymphoid cells (ALS) for diminishing cell-mediated immune responses. Such sera have been shown to prolong the survival of skin grafts across an H-2 barrier in adult mice (11–13). Rabbit antisera against rat thymus were reported by Anigstein *et al.* (14, 15) to increase the size of sarcoma 180 implants or growth of a transplantable mammary adenocarcinoma in mice. The incidence of adenovirus type 12 tumors in two strains of mice was increased by injections of ALS to an extent comparable with the increase following neonatal thymectomy (5). This system is known to have a virus-specific antigen eliciting a cell-mediated immune response. It was therefore of interest to compare the effects of ALS and neonatal thymectomy in other systems such as the induction of tumors by polyoma virus in different strains of mice and the induction of virus leukemia. The results of such experiments are reported in this paper.

Materials and Methods. Mice. Newborn mice of strains C57BL/Ka, BALB/c and C3Hf/He were obtained from the breeding colonies in the Carcinogenesis Section, National Cancer Institute, or the general breeding colony of the National Institutes of Health, Bethesda. Some experiments on leukemogenesis in BALB/c mice were carried out with mice from an inbred colony at the National Institute for Medical Research, London, England.

Virus strains. The LID_1 strain of polyoma virus (16), propagated and titrated in mouse embryo cell cultures, was received from Dr. R. C. Ting. Newborn mice of several strains were inoculated subcutaneously with 0.05 ml virus containing 6×10^5 pfu polyoma virus. Plasma from MLV infected mice (MLV, Melpar batch MVPC 28) was obtained from Dr. J. B. Moloney; 0.1 ml of a 1:10 dilution of stock virus was used as inoculum. The original undiluted MLV and sonicates of some induced reticulum cell sarcomas were tested for the presence of murine sarcoma viruses (MSV) by intramuscular inoculation into newborn BALB/c and C57BL mice; no tumors were observed, although generalized lymphocytic leukemias appeared after about 3 months.

Antilymphocyte sera. These were prepared in rabbits of the New Zealand strain by two intravenous injections of 10^9 thymus cells from CBA mice at an interval of 14 days and bleeding 7 days after the second injection. Before use sera were decomplemented by heating at 56°C for 20 min and absorbed with washed erythrocytes from the same strain of mice as used for subsequent injection. Absorption was repeated until the erythrocytes were no longer agglutinated. In some experiments the IgG fraction of ALS (ALS-IgG) prepared by chromatography was used in place of whole ALS. Before using ALS-IgG newborn mice were made tolerant to rabbit IgG (RGG) by two injections of 5 mg on day 1 and day 10 after birth. The RGG and ALS-IgG were centrifuged at 12,000*g* for 20 min before injection to remove aggregates of denatured protein. Subsequently mice were given 0.2 ml ALS-IgG, containing 5 mg of protein as described in the text. Controls received comparable injections of RGG.

Examination for tumors. Animals were examined twice weekly for appearance of tumors. These were allowed to grow to approximately 3 cm diameter before the animals were killed: all tumors were fixed in Tellyesniczky's solution and sections stained with hematoxylin and eosin for histological examination.

Results. Polyoma virus oncogenesis in C57BL/Ka mice. Twenty-two newborn mice of this strain were infected with polyoma virus within 24 hours of birth and given 0.1 ml of ALS subcutaneously on the following day and twice weekly for 3–4 weeks. The majority of treated mice were smaller than controls, five showing severe runting comparable to that previously seen in neonatally thymectomized mice of the same strain in this laboratory (3). Only 15 mice survived longer than 1 month, and 10 longer than 2 months; all of these developed bilateral parotid gland tumors at 2–2.5 months of age. None of 28 controls infected with polyoma virus under the same conditions developed tumors, even though kept under observation for 9 months.

Polyoma virus oncogenesis in C3Hf/He mice. Twenty-three newborn mice of this strain were infected with polyoma virus within 24 hours of birth and given ALS on the second day and twice weekly thereafter for 3–4 weeks. Treated animals showed retarded growth, 5 of them with severe runting. Only 7 animals survived longer than 2 months and all developed parotid gland tumors between 2 months and 4.5 months of age. In addition, mammary gland and multiple hair follicle tumors were observed. None of 9 control animals infected with polyoma virus and kept under observation for 9 months developed a tumor.

Effects of ALS and of thymectomy on MLV leukemogenesis in BALB/c mice. Thirty-three newborn BALB/c mice were injected with 0.1 ml ALS subcutaneously on the day of birth and twice weekly for 3–4 weeks. Three weeks after birth they were inoculated with MLV intraperitoneally. Several mice receiving ALS died and others were smaller than controls; the weights of 10 survivors at 6 weeks were 14.7 gm (8.5–22 gm) as compared with 21.0 gm (18.5–24.5 gm) in controls. White blood cell counts of treated animals were low (approximately 5,000/mm^3), the deficiency affecting principally lymphocytes; 5–10% of the circulating leukocytes were large blast-like mononuclear cells.

Thirteen of the 19 mice surviving treatment with ALS developed subcutaneous nodules, beginning 21 days after infection with MLV. These animals were submitted to autopsy 46–52 days after infection. At necropsy the prominent finding was a localized soft whitish growth in the subcutaneous connective tissue, measuring on the average 32 × 13 × 13 mm. Usually the liver was enlarged and friable and the spleen was enlarged and nodular. When the tissues were examined microscopically, extensions of the lesions were usually seen into liver, spleen, and some lymph nodes, principally mesenteric nodes. There was slight to moderate hemapoiesis in the spleens and livers of some treated mice. The thymus was usually atrophic. All but one of the localized neoplastic growths could be classified as reticulum cell sarcomas, type A of Dunn (17) (Fig. 1). One was thought to be either a type B reticulum cell sarcoma or a mixed monocytic-myeloid leukemia. Two of the reticulum cell sarcomas were found to grow progressively following transplantation into syngenic BALB/c mice. Of the 6 animals treated with ALS and infected with MLV not showing reticulum cell sarcomas, 5 developed generalized lymphoid leukemia 3–3.5 months after infection (Fig. 2).

None of 35 controls infected with the same stock of MLV at 21 days developed a reticulum cell sarcoma. Seventeen of 35 survivors kept under observation for 8 months developed generalized lymphocytic leukemia beginning 3 months after infection.

In order to check that the early appearance of localized subcutaneous reticulum cell sarcomas was not due to a peculiarity of the particular batch of antiserum used, or was influenced by an immune reaction to some component of rabbit serum, further experiments were carried out in BALB/c mice made tolerant to rabbit IgG given subcutaneous injections of ALS-IgG (prepared from another ALS pool) on day 14, 22, and 29, after birth, and infected with MLV by intraperitoneal injection on day 21 after birth. Four of 14 mice developed subcutaneous reticulum cell sarcomas between 4 and 6 weeks after infection with MLV. Six of the remaining 10 mice developed generalized leukemia between 3.5 and 4.5 months of observation.

Discussion. Comparison of ALS and early thymectomy on polyoma virus oncogenesis. Previous studies in this laboratory have shown that C57BL/Ka mice are very resistant to polyoma virus oncogenesis (3). Of 52 mice infected with the LID_1 strain of polyoma virus between 4 and 7 days after birth one developed a tumor. In contrast, 33 out of 45 (73%) of mice thymectomized at 3 days and infected with 2×10^6 pfu of polyoma virus at the same age developed tumors. In the present study, all of 10 survivors infected with 6×10^5 pfu of LID_1 polyoma virus and treated with ALS developed bilateral parotid tumors whereas none of 28 controls infected at the same time developed tumors.

In previous studies with C3H strain (3), 2 of 20 control mice infected with LID_1

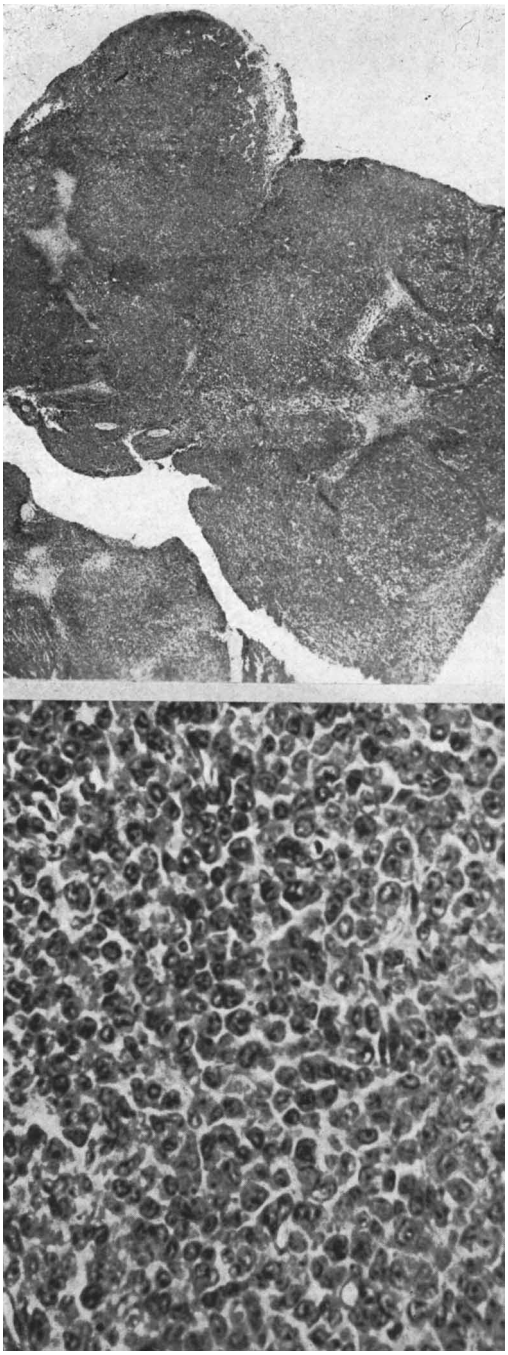

FIG. 1. Subcutaneous reticulum cell sarcoma on back of BALB/c male, measuring 36 × 14 × 14 mm at necropsy (25 days after injection of MLV intraperitoneally). Mouse received ALS × 7 beginning at 24 hours of age. See text. Upper photo, ×19; lower photo, ×340. Hematoxylin and eosin.

strain of polyoma virus between 4 and 16 days after birth developed tumors, whereas 25 out of 34 mice thymectomized at 3 days (74%) developed tumors. In the present study, none of 9 control C3H mice infected with LID_1 strain of polyoma virus neonatally showed tumors whereas all of 7 animals surviving infection and ALS treatment developed tumors. Tumors in controls were confined to one salivary gland, whereas some of these in thymectomized animals or animals treated with ALS were multiple, involving mammary glands, subcutaneous connective tissues, and hair follicles. It is therefore clear that either

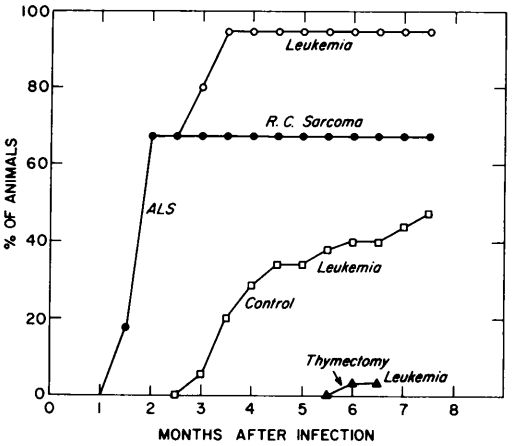

FIG. 2. Frequency and time to necropsy of lymphoid neoplasms in BALB/c mice. All were injected at 21 days of age intraperitoneally with MLV. The ALS-treated group developed early reticulum cell sarcomas (RCS) locally at the site of ALS injections. Those mice remaining free of RCS developed a generalized lymphocytic leukemia. Non ALS-treated (controls) developed generalized lymphocytic leukemia while the 3-day thymectomized group resisted the leukemogenic effects of MLV.

early thymectomy or treatment with ALS is highly effective in increasing polyoma virus oncogenesis in relatively resistant strains of mice. The results suggest that ALS treatment may be even more efficient than 3-day thymectomy in increasing tumor yield, although further experiments carried out under exactly comparable conditions would be required to be sure that this is so. In any case, ALS has some advantages over thymectomy in studies of oncogenesis. The dosage and timing of the immunosuppressant effect can be better con-

trolled with antiserum, and simple injections are easier to perform than surgery, especially in animals such as hamsters and rabbits.

The doses of ALS used in the present experiments also produced depletion of lymphoid cells, runting, and death of many experimental animals. However, other results suggest that much smaller doses of antisera which do not produce runting can be effective in increasing virus oncogenesis. Thus, Allison *et al.* (5) found that three doses of ALS were sufficient to increase adenovirus type 12 oncogenesis in mice. Jooste[1] observed that injections of ALS in CBA mice on the day of birth and day 11 prolonged the survival of skin grafts across an H-2 barrier made on day 21. Russe and Crowle (18) have also found that delayed hypersensitivity to protein antigens is abolished or depressed by injections of ALS into newborn mice. The efficacy of ALS in very young animals is presumably due to two factors: the ease with which it is possible to neutralize the relatively small number of lymphoid cells which can normally be mobilized in cell-mediated immune responses, and the fact that in very young animals tolerance to rabbit IgG is readily induced, thereby prolonging the survival and efect of ALS-IgG. The ALS circulating throughout the body is presumably able to reach any thymus-dependent cells that have already seeded out from that organ.

In general, these and other results (5) support the generalization made (15) that in animals infected immediately after birth with polyoma virus, adenovirus type 12 and simian virus 40, tumor yields can be consistently increased by immunosuppressive procedures. Hence oncogenesis by these viruses is normally limited by host immune responses, from which it can be concluded that neonatal infection with these viruses does not result in tolerance.

Effects on MLV virus leukemogenesis. The experiments reported in this paper confirm previous evidence (8) that early thymectomy decreases the incidence of the typical lymphocytic leukemias following infection with MLV. In contrast, treatment with ALS increases the yield of tumors after infection of 3-week-old animals with MLV. Moreover, the pathology of the tumors is strikingly altered, with the early appearance of subcutaneous reticulum cell sarcomas at the site of injection of ALS. It seems that three factors may contribute to this remarkable and unexpected finding. First, the nonlymphoid cells of the thymus which facilitate neoplastic conversion by MLV survive treatment with ALS, whereas they are eliminated by thymectomy. Second, the immunosuppressive effect of the ALS may allow the survival of cells soon converted to neoplasia by the virus but so highly antigenic that they would be eliminated in the intact animal. The MLV is known to induce resistance against transplantation of lymphoma cells carrying the corresponding antigen in BALB/c mice (19). Third, ALS stimulates the proliferation of primitive lymphoid cells as shown by increased incorporation of labeled thymidine into their DNA, and the appearance of blast cells in peripheral blood.[2] The combined effects of blast-cell proliferation and MLV may have led to the induction of reticulum cell sarcomas. The fact that these were observed subcutaneously suggests that circulating cells, localizing at the sites of injection of ALS, were involved. Reticulum cell sarcomas are usually a relatively rare and late consequence of exposure to MLV (20) or in spontaneous occurrence.

It should be possible to determine the relative importance of the immunosuppressive and blast-cell-inducing actions of ALS by observing the effects of the serum on mice neonatally infected with MLV and tolerant to the infection, in which the former effect would presumably be negligible. Experiments are in progress to test this point. Theoretically, it might have been expected that ALS, acting as it does on lymphoid cells, would delay or reduce the incidence of generalized leukemia. No such effect was observed, but in our experiments administration of ALS was discontinued 1 week after administration of MLV; other regimens might delay or impair leukemogenesis. In general, the leukemias provide a situation in which the effect of ALS treatment and thymectomy are clearly

[1] Jooste, S., unpublished data.

[2] Gillman, T. and Allison, A. C., unpublished observations.

different, and each experimental manipulation helps to throw light on the underlying biological processes.

Summary. Repeated injections of rabbit antiserum against the lymphoid cells of mouse thymus (ALS) were found to increase markedly the evidence of tumors in resistant strains of mice neonatally infected with polyoma virus. In this system the effects of ALS are similar to those of early thymectomy. The BALB/c mice receiving repeated injections of ALS and infected intraperitoneally with Moloney leukemogenic virus (MLV) when 21 days old showed an increased incidence of lymphoid neoplasms as compared to that in untreated controls infected at the same age. The majority of tumors in the treated animals were reticulum cell sarcomas, type A, developing subcutaneously at the site of injection of the ALS 3–6 weeks after infection with MLV. Early thymectomy markedly decreased the incidence of leukemias in BALB/c mice infected with MLV when 3 weeks old. In this system the effects of ALS are the opposite of those produced by thymectomy. It is concluded that the thymic elements required for neoplastic conversion by MLV are not eliminated by ALS, which may alter the pathology of the induced tumors by a combination of immunosuppression and capacity to stimulate the proliferation of primitive lymphoid cells.

Grateful acknowledgement is made to Dr. Mearl Stanton, National Cancer Institute, for help in diagnosis of the reticular neoplasms and to Mr. Britton Smith for technical help.

1. Hellström, K. E. and Möller, G., Progr. Allergy, **9**, 158 (1965).
2. Old, L. J. and Boyse, E. A., Federation Proc., **24**, 1009 (1965).
3. Law, L. W., Cancer Res. **26**, 551 (1966).
4. Allison, A. C. and Taylor, R. B., Cancer Res., 1967, in press.
5. Allison, A. C., Berman, L. H., and Levey, R. H., Nature, 1967, in press.
6. Martinez, C., Nature **203**, 1188 (1964).
7. Gross, L., Proc. Soc. Exptl. Biol. Med., **100**, 325 (1959).
8. Moloney, J. B., Federation Proc. **21**, 19–31 (1962)
9. Law, L. W. and Potter, M., Proc. Natl. Acad. Sci., U. S. **42**, 160 (1956).
10. Carnes, W. H., Kaplan, H. W., Brown, M. G., and Hirsch, B. B., Cancer Res. **16**, 429 (1956).
11. Woodruff, M. F. A. and Anderson, N. F., Ann. N. Y. Acad. Sci., **120**, 119 (1964).
12. Monaco, A. P., Wood, M. C., Gray, J. G., Russell, P. S., J. Immunol. **96**, 229 (1966).
13. Levey, R. H. and Medawar, P. B., Proc. Natl. Acad. Sci., U. S., **56**, 1130 (1966).
14. Anigstein, L., Anigstein, D. M., and Rennels, E. G., Texas Rept. Biol. Med. **23**, 705 (1965).
15. Anigstein, L., Anigstein, D. L., Rennels, E. G., and O'Steen, W. K., Cancer Res. **26**, 1867 (1966).
16. Law, L. W. and Ting, R. C., Proc. Soc. Exptl. Biol. Med. **119**, 823 (1965).
17. Dunn, T. B., J. Natl. Cancer Inst. **14**, 1281 (1954).
18. Russe, H. P, and Crowle, A. J., J. Immunol. **94**, 74 (1965).
19. Glynn, J. P., Bianco, A. R., and Goldin, A., Cancer Res. **24**, 502 (1964).
20. Metcalf, D., Wiadrowski, M., and Bradley, R., Natl. Cancer Inst. Monograph **22**, 571 (1966).

Received August 28, 1967. P.S.E.B.M., 1968, Vol. 127.

Effect of Plasma Cell Tumor on Antibody Production by Mouse Spleen Cells (32658)

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Circulating antibody was shown to be quantitatively reduced in C_3H mice bearing the transmissible plasma cell tumor, X5563, following a primary inoculation with sheep erythrocytes (1). This effect might be due in part to a reduction in the numbers of lymphoid cells synthesizing antibody; to a competitive mechanism depleting the protein precursors of the antibody; to an abnormal utilization of the globulin molecules after their synthesis; or to some combination of these conditions. The present experiments were car-