ously reported that organ PFC responses in mice chronically exposed to cigarette smoke were impaired[9,10] and the depression in the serum antibody levels shown here indicates that the decrease in the numbers of antibody-forming cells in the organs is not compensated for by an increase in antibody produced per cell, or by antibody-producing cells in an organ not examined.

The direct effect of cigarette smoking on antibody levels appears negligible, as judged by the failure of vigorous treatments with smoke solutions to inactivate either the haemagglutinating or haemolytic antibody. The antibody-producing cells exhibited a degree of sensitivity to high concentrations of cigarette smoke solutions, and the direct effect of cigarette smoke on plasma cells in the respiratory system must be considered as a possible factor moderating immune function in the lungs of cigarette smokers. However the direct effect of cigarette smoke on antibody-forming cells elsewhere in the body is probably negligible.

Antibody response of mice chronically exposed to cigarette smoke[a]

	Smoke exposed	Control
Haemolysin	0.22 ± 0.14	1.25 ± 0.45
Haemagglutinin	1.45 ± 0.24	3.40 ± 0.67

[a] Mice were exposed to cigarette smoke for 19 weeks and inoculated intratracheally with SRBC. Each result is the mean ± S.E. of the $\log_2$ serum titres of groups of 9–10 mice, measured 1 week after the inoculation. The differences between the titres of the smoke-exposed and control mice were significant as judged by Student's *t*-test ($p < 0.05$).

Zusammenfassung. Die Bildung von Antikörpern im Serum, hervorgerufen durch Inokulation von Schaferythrocyten in der Mäusetrachea, wurde durch chronische Inhalation von Zigarettenrauch vermindert.

W. R. THOMAS, P. G. HOLT[15] and D. KEAST[16]

Department of Microbiology, University of Western Australia, Perth Medical Centre, Shenton Park 6008 (Western Australia), 1 July 1974.

[15] To whom requests for reprints should be addressed.

[16] Acknowledgments. The work was supported by a grant from the Australian Tobacco Research Foundation. WRT is in receipt of a C.S.I.R.O. Post-graduate studentship. The authors wish to thank Miss LYN ROBERTS for her skilled technical assistance.

Antiserum Inactivation of Hematopoietic Colony Forming Stem Cells from Mice with Rauscher Leukemia

Previous reports from our laboratory and others have indicated that the pluripotent hematopoietic colony forming stem cell (CFU-S) in the mouse spleen is a possible target cell for Rauscher leukemia virus[1–3]. Injection of the Rauscher virus (RLV) has been shown to effect this cell compartment in several ways, including inducing an oscillatory change in the proportion of spleen cells which are in the CFU-S compartment[4] and an eventual overall increase in the absolute number of CFU-S concomitant with the splenomegaly aspect of the disease[3–6]. Other studies concerned with the transplantation of CFU-S from Rauscher leukemic mice have shown the transplantation efficiency (f-factor) to be lowered by the leukemia, suggesting that at least a portion of that compartment was directly affected by the virus and may consist of transformed cells[1]. In order to obtain further evidence for the possible existence of transformed cells among the CFU-S of the Rauscher leukemic mouse, we have carried out additional experiments to determine if a

[1] J. P. OKUNEWICK and E. L. PHILLIPS, Blood *42*, 885 (1973).
[2] E. J. P. BROMMER, *The Role of the Stem Cell in Rauscher Leukemia* (Radiobiological Institute, TNO, Holland).
[3] H. J. SEIDEL, Z. Kresbsforsch. *79*, 123 (1973).
[4] A. M. MARKOE, J. P. OKUNEWICK, P. ERHARD and E. PHILLIPS, J. natn. Cancer Inst. *50*, 449 (1973).
[5] A. M. WU, M. PARAN and R. C. GALLO, Blood *40*, 951 (1972).
[6] J. P. OKUNEWICK, E. L. PHILLIPS and P. ERHARD, Blood *49*, 1101 (1972).

Effect of antiserum incubation on the colony forming stem cells of the Rauscher leukemic mouse spleen

Incubation medium	No. of colonies formed per 10^6 transplanted cells at 8 days after transplantation		
	8-Day leukemic mouse donor	21-Day leukemic mouse donor	Normal mouse donor
Hanks' solution	37.5 ± 3.9[a]	13.4 ± 1.3	120.8 ± 5.9
Normal serum	38.6 ± 3.1	13.1 ± 1.3	120.8 ± 9.8
Antiserum	26.0 ± 2.9	5.7 ± 1.0	135.9 ± 7.1
Inhibition of colony formation by antiserum (%)	32	56	none

[a] Mean ± 1 standard error of 17 or more separate determinations.

direct effect of the virus on stem cells could be detected by other, independent, parameters. The present report gives our initial results on one of these, the question of whether any of the cells in this compartment might carry the leukemia antigen and therefore be sensitive to antiserum prepared against the diesease.

Materials and methods. Antiserum to Rauscher leukemia was prepared using the same method employed by THOMSON and AXELRAD[7] in their demonstration of antiserum sensitive cells among the CFU-S of Friend leukemic mice. Mice of the same sex and syngeneic to the strain to be studied (female SJL/J) were injected with RLV. 2 weeks later these mice were sacrificed and their spleens removed and homogenized. This suspension, which contained both transformed cells and free virus, was treated with formalin at 4 °C for 24 h in order to inactivate its leukemogenic potential. The suspension was then centrifuged, the precipitate resuspended in conjunction with Freund's adjuvant and injected s.c. into normal SJL/J mice of the same sex. This procedure was repeated weekly for 9 weeks, after which the serum was collected from these recipients.

Separately, a second set of 6 mice (8-week-old female SJL/J) which were to serve as a source of CFU-S for study were injected i.p. with 50 SED 50/14 units of RLV[1]. At 8 days and again at 21 days after virus injection 3 of these mice were sacrificed, their spleens removed and the spleen cells suspended in Hanks' solution in the amount of approximately 0.2 ml of packed cells per ml of solution. To each of three 1 ml aliquots of the suspension were added 1 ml of antiserum, normal SJL/J serum, or Hanks' solution respectively. The suspensions were then incubated at 37 °C for 30 min in a shaking water bath. For control purposes the identical procedure was also carried out using an additional set of mice to obtain normal CFU-S from non-leukemic SJL/J mice of the same age and sex.

Assays for the number of viable CFU-S were carried out using the TILL and MCCULLOCH[8] technique, with the recipient assay mice being irradiated with 950 R, as described previously[1,4,6], to suppress their endogenous CFU. A minimum of 17 mice were used for the CFU-S assay of each of the above treated spleen cell suspensions. Into these, after incubation, 0.5 ml volumes of the cell suspensions were injected via the tail vein, containing in the case of the leukemic cells between 2.5 and 10×10^5 cells, and in the case of the normal cells between 1.25 and 1.75×10^5 cells. 8 days later these recipients were sacrificed, their spleens removed, fixed in Bouin's solution, the colonies counted and the number of viable CFU-S in the injectate determined.

Results. The effect of incubation before transplantation of the spleen cell suspension containing the CFU-S with the Rauscher-leukemia-antiserum, of incubation with normal serum, and of incubation with Hanks' solution are shown in the Table for both normal and leukemic mice. Values for CFU-S inactivation for 8 and 21 day leukemic mice and for the control animals are given. We have reported previously that the periods of 1 and 3 weeks after RLV injections are points of maximum depression in the proportion of CFU-S for the virus titer used[4]. The present results are consistent with those findings. Relative to the number of colonies formed per 10^6 injected spleen cells within each of the 3 groups of the Table, there was no significant difference in the number of colonies formed when incubation in Hanks' solution was compared with that in normal serum. Incubation with the antiserum, in contrast, resulted in significant depression of colony forming ability at the levels of better than $\alpha = 0.01$ for both the 8- and 21-day leukemic mice, while at the same time the difference seen for incubation of the normal spleen cells with antiserum was not significantly different with respect to the normal serum or Hanks' solution treatment at the level of $\alpha = 0.10$ and was in the opposite direction from that for the leukemic cells. The results therefore show that, similar to the findings of THOMSON and AXELRAD[7] for Friend leukemia the CFU-S compartment of the Rauscher leukemic mouse spleen contains cells which can be inactivated by antiserum prepared against the disease in syngeneic mice.

Discussion. The findings of this study provide additional evidence to support the thesis that the pluripotent hematopoietic stem cell may be a target cell for Rauscher leukemia virus. They also indicate that the CFU-S compartment in the viral leukemic mouse may be made up of at least two components. From the present study we would tentatively classify these as a CFU-S component of cells having the tumor antigen and therefore capable of being inactivated by the antiserum, and as a CFU-S component cells either lacking the antigen or with insufficient antigen to allow for inactivation. Whether or not this latter group consists purely of normal cells or can be further broken down into a mixture of normal and transformed cells remains an open issue. However, relative to the main question of the study several different lines of previous evidence now implicate the pluripotent hematopoietic stem cell as a target cell for Rauscher leukemia virus. These include, in addition to the present findings, the proliferative response of hematopoietic stem cells to Rauscher leukemia[3–6], the pluripotent differentiation capabilities of those cells following their transplantation from Rauscher leukemic mice[2,5,9], their radioprotective capability[2,10] and their loss of transplantation efficiency[1]. Of these, previously only the effect on transplantation efficiency could have been considered as truly direct evidence of leukemic transformation of the stem cell. The inclusion of inactivation of cells in the CFU-S compartment by Rauscher tumor antiserum adds one more line of direct evidence suggesting that hematopoietic stem cells should be considered as target cells for leukemia viruses.

Zusammenfassung. Nachweis, dass pluripotente Stammzellen von Mäusen mit Rauscher-Leukämie teilweise durch Rauscher-Virus-Antiserum inaktiviert werden. Ein Teil der pluripotenten Stammzellen scheint vom Virus befallen zu sein.

J. P. OKUNEWICK, E. L. PHILLIPS and B. J. BROZOVICH[11]

Cellular and Radiation Biology Laboratories, Radiation Therapy Research Center, Allegheny General Hospital, 320 East North Avenue, *Pittsburgh (Pennsylvania 15212, USA), 28 May 1974.*

[7] S. THOMSON and A. A. AXELRAD, Cancer Res. *28*, 2105 (1968).
[8] J. E. TILL and E. A. MCCULLOCH, Radiation Res. *16*, 822 (1962).
[9] J. P. OKUNEWICK, A. M. MARKOE, P. ERHARD and E. L. PHILLIPS, J. Lab. clin. Med. *81*, 489 (1973).
[10] J. P. OKUNEWICK and P. ERHARD, Radiation Res. *53*, 241 (1973).
[11] Supported in part by NIH-NCI Grant No. 1P02 CA 10438, by Grant No. HRSF N-32 of the United Fund of Pittsburgh, and by U.S. Atomic Energy Commission Contract No. AT (11-1) 3097.