

Fig. 1. PHA response in patients with glomerulonephritis with and without immunosuppression. AGN, acute glomerulonephritis; CGN, chronic glomerulonephritis.

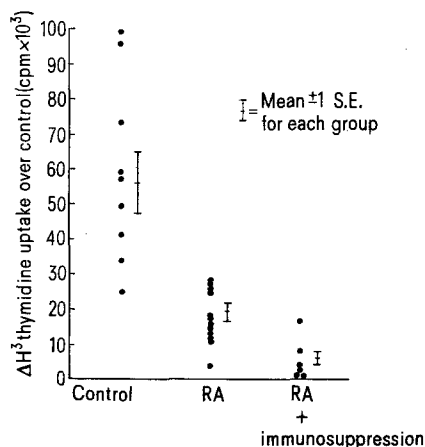


Fig. 2. PHA response in rheumatoid arthritis (RA) with and without immunosuppression.

Results. Patients with RA had a lower PHA response than controls (Figure 1). Immunosuppressed patients with RA demonstrated an even lower response than RA patients. These differences were significant at the 95% level (paired *t*-test).

Lymphocytes from patients with AGN responded in the same manner as the control group (Figure 2). The response of lymphocytes from CGN patients was slightly lowered but this difference was not statistically significant.

Immunosuppressed CGN patients showed a much lower response than controls, AGN and CGN groups at the 99% level (paired *t*-test). No correlation was observed between the lowered response and the mode of immunosuppression used.

Discussion. This study demonstrates that patients with RA have a significantly decreased lymphocyte response to PHA whereas patients with AGN and CGN do not differ significantly from controls. The consistent decrease in lymphocyte response in patients on immunosuppressive agents is especially important in light of the recent literature suggesting an increase in cellular immunity to certain antigens in renal disease⁷. The effect of immunosuppression can be observed as a decrease in lymphocyte response to PHA. This test may be of value in monitoring patients on immunosuppressive therapy⁸.

Résumé. La transformation lymphocytaire en phytohemagglutinine est étudiée chez des individus normaux,

aussi bien que chez des malades souffrant de glomérulonephrites aiguës et chroniques ainsi que de polyarthrite rhumatoïde. La réponse des glomérulonephritiques fut conforme aux contrôles, les polyarthritiques eurent des réponses affaiblies. La transformation lymphocytaire est fortement réduite chez les patients sous immunosuppression.

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Stem Cells in the Peripheral Blood of Rauscher Leukemic Mice

Stem cells circulate in the peripheral blood of mice¹ and can easily be detected by the spleen colony assay² (review see ³). Recently it has been shown that stem cells in the spleen and the bone marrow, measured as colony-forming units (CFU), are affected by the Rauscher virus^{4,5} but no experiments have been performed with peripheral blood cells in any virus-induced leukemia to my knowledge. In this report, data are given for CBA mice infected with Rauscher virus.

Groups of 4-7 female mice (8-10 weeks old) were bled from the retro-orbital sinus 2, 5 or 13 days after infection and 0.2-0.35 ml of blood were injected into lethally

irradiated (800 R) isogenic recipients (10 per group). 23 days after infection, blood from a single mouse with erythroblastosis was taken, diluted with saline and 0.2 ml containing 0.04 ml of blood was injected. Blood from 2

¹ J. W. GOODMAN and G. S. HODGSON, *Blood* 19, 702 (1962).

² L. COLE, *Am. J. Physiol.* 204, 265 (1963).

³ D. METCALF and M. A. S. MOORE, *Haemopoietic Cells* (North Holland Publishing Co., Amsterdam, London 1971).

⁴ H. J. SEIDEL, *Vth Int. Symp. Comp. Leukemia Res.* 1971. *Biblph. Haemat.*, in press.

⁵ H. J. SEIDEL, *Z. Krebsforsch.*, 79, 123 (1973).

control groups at the beginning and the end of series was studied in the same way. 9 days after irradiation and injection of the peripheral blood cells, the spleens of the recipient mice were fixed in Bouin's solution and the colonies were counted.

The results are given in the Table. A depression of the CFU content of peripheral blood was observed 2 days after infection. The same phenomenon had been found

previously in the bone marrow and the spleen⁵. After 5 and 13 days an increase of CFU's/ml blood occurred. The peripheral blood cell count of the donor mice varied between 4,000 and 7,300 nucleated cells/mm³ without more than 2% erythroblasts, but with an increasing percentage of lymphocytes. The animal 23 days after infection had severe erythroblastosis and a 50-fold rise in CFU's/ml blood.

These experiments are being extended in current studies in the laboratory with special reference to the leukemic or normal status of these CFU's, using the F₁-hybrid system⁶.

Zusammenfassung. CBA Mäuse zeigen nach der Infektion mit dem Rauscher Virus einen starken Anstieg hämopoetischer Stammzellen («colony-forming units») im peripheren Blut. Nach 21 Tagen wird im Zustand der peripheren Erythroblastose eine 50-fache Zunahme gefunden.

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Rauscher virus leukemia
CFU's in the peripheral blood

Days after infection	Injected blood volume (ml)	Average colonies counted per spleen	CFU/ml blood
Control I	0.3	2.6 ± 0.3 ^b	7.8 ± 1.0
2	0.35	0	0
5	0.2	16.6 ± 1.7	83 ± 8.5
13	0.2	20.2 ± 0.8	101 ± 4
23 ^a	0.04	18.6 ± 1.2	465 ± 30
Control II	0.3	2.8 ± 0.4	8.4 ± 1.3

^a Blood cell count 54,000 cells/mm³ (83% erythroblasts). ^b ± SEM.

⁶ S. THOMSON and A. A. AXELRAD, *Cancer Res.* 28, 2105 (1968).

⁷ Supported by the Deutsche Forschungsgemeinschaft.

Interactions of Anti-DNA Antibodies with Dinitrophenyl- and other Hapten-Protein-Conjugates

Several myeloma proteins in the BALB/c strain of mice^{1,2} and in man³ have been reported to react with dinitrophenyl (DNP) and with compounds, which seem to be structurally unrelated to DNP, such as 5-acetyluracil, purine-6-oyl and DNA. Conventionally induced anti-DNP antibodies have been shown to give the same unexpected crossreactions^{1,4-6}. The crossreactivity of antibodies to DNA with DNP, however, has not yet been investigated.

The purpose of the present report was therefore to study the interactions of antibodies to DNA as found in patients with systemic lupus erythematosus^{7,8} (SLE) with DNP- and various other hapten-protein-conjugates, including 5-acetyluracil, purine-6-oyl, nucleosides and nucleotides coupled to bovine serum albumin (BSA).

As some of the previously mentioned myeloma proteins with anti-DNP specificity seemed to react only with DNP-protein-conjugates, not with the free haptens³, we used a radioimmunoassay in order to demonstrate crossreactions of anti-DNA antibodies. This method is applicable to a measurement of the relative binding affinities of antibodies for complete antigens as well as for haptens.

Material and methods. Sera of 4 patients with SLE were investigated. The patients IgG isolated by ion-exchange chromatography was coupled to bromoacetylcellulose (BAC) according to ROBBINS et al.⁹. DNP-BSA was labeled with ¹²⁵I by the iodine monochloride method described by McFARLANE¹⁰. The reaction of the insolubilized IgG-BAC with ¹²⁵I-DNP-BSA was taken as 100%

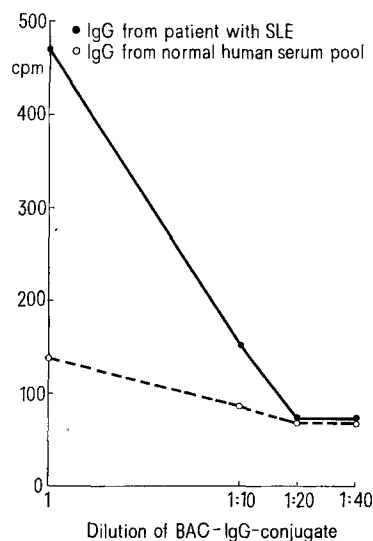


Fig. 1. Binding of ¹²⁵I-DNP-BSA to IgG-BAC from a patient with SLE (filled circles) and to IgG-BAC from a normal human serum pool.

¹ D. SCHUBERT, A. JOBE and M. COHN, *Nature, Lond.* 220, 882 (1968).

² D. SCHUBERT, A. ROMAN and N. COHN, *Nature, Lond.* 225, 154 (1970).

³ W. RIESEN and A. MORELL, *Immunochemistry* 9, 979 (1972).

⁴ M. A. SMITH and M. POTTER, *Fedn. Proc.* 28, 819 (1969).

⁵ B. J. UNDERDOWN and H. N. EISEN, *J. Immun.* 106, 1431 (1971).

⁶ W. RIESEN, S. CARREL, V. CASTEL and A. MORELL, *Abstr. Commun. Meeting Fed. Eur. Biochem. Soc.* 8, 688 (1972).

⁷ M. SELIGMANN, *C.R. Acad. Sci., Paris* 245, 243 (1957).

⁸ W. C. ROBBINS, H. R. HOLAMN, H. DEICHER and H. G. KUNKEL, *Proc. Soc. exp. Biol. Med.* 96, 575 (1957).

⁹ J. B. ROBBINS, J. HAIMOVICH and M. SELA, *Immunochemistry* 4, 11 (1967).

¹⁰ A. S. McFARLANE, *Nature, Lond.* 182, 53 (1958).