

elsewhere, the intracardiac inoculation of animals with SV₄₀ stimulated the growth of transplantable carcinogen-induced tumours⁹. This observation, as well as the capacity of the above virus to intensify the carcinogenicity of DMBA in new-born hamsters¹⁰, may have something to do with the immuno-suppressive property of SV₄₀.

An immunosuppressive effect of DNA-containing viruses seems to account for the fact that tumours induced by them grow in spite of the existence of strong transplantation antigens on their surface.

A preliminary experiment in which sheep erythrocytes were injected to hamsters 9 days before inoculated with SV₄₀ has shown a marked suppression of antibody formation against the above erythrocytes.

Выводы. Золотистых хомячков заражали вирусами SV₄₀ или адено типа 16. Через 9 дней после заражения им вводили живой парагриппозный вирус Сендай. У этих жи-

вотных наблюдалось подавление антителообразования к вирусу Сендай по сравнению с контрольными.

В связи с полученными данными кратко обсуждается проблема иммунодепрессивного действия онкогенных вирусов.

VERA P. HAMBURG, OLGA E. SCHERBAKOVA
and G. J. SVET-MOLDAVSKY

*Laboratory of Virology,
Institute of Experimental and Clinical Oncology,
USSR Academy of Medical Science,
Moscow M-478 (USSR), 8 September 1969.*

⁹ G. J. SVET-MOLDAVSKY, V. P. HAMBURG and A. L. LIOZNER, Abstr. of 9th Int. Cancer Congr., Tokyo (1967), p. 335.

¹⁰ V. P. HAMBURG and G. J. SVET-MOLDAVSKY, *Nature, Lond.* 215, 5107 (1967).

Phagocytosis of ¹⁴C Dinitrophenyl Poly L-Lysine by Peritoneal Exudate Cells from Guinea-Pigs

Guinea-pigs of strain II and strain XIII respond differently to synthetic antigens, as demonstrated by the experiments of LEVINE and BENACERRAF¹, and BEN-EFFRAIM, FUCHS and SELA². Hapten conjugates of poly L-lysine, for example, elicit an immune response in strain II, but fail to do so in strain XIII; this facility being a dominant autosomal unigenic trait. It is pertinent to the question of whether or not phagocytosis is involved in antibody synthesis³⁻⁵ to determine if these strains also differ in their ability to process poly L-lysine conjugates this way. A direct correspondence between phagocytic ability and antibody synthesis would obviously accord with the participation of both phagocytic and lymphocytic cells in the immune response. Were no difference to be found in the phagocytosis of the antigen by responsive and non-responsive strains, one must infer, either that the state of unresponsiveness is attributable to a post-phagocytic block in antibody synthesis, or else phagocytosis has no relevance to the immune response.

Data are presented that demonstrate the ability of peritoneal exudate cells from strain II and XIII guinea-pigs to phagocytize ¹⁴C dinitrophenyl poly L-lysine (¹⁴C-DNP-PLL). The DNP-PLL used was prepared as described by LEVINE and BENACERRAF⁶. PLL was kindly given by Prof. P. DOTY, Harvard University, and had an average degree of polymerization of 398. Uniformly labelled ¹⁴C-DNP (Amersham; 34 µc/mg) was employed as the hapten. On the average, twelve ¹⁴C-DNP groups were substituted per molecule of PLL to form ¹⁴C-DNP₁₂-PLL₃₉₈.

Male guinea-pigs (approx. 800 g) were injected i.p. with 20 ml 1% sodium chloride and 0.1% glycogen, 3 days before being sacrificed. 4 h prior to sacrifice, 2.6 mg ¹⁴C-DNP₁₂-PLL₃₉₈ was administered i.p. The peritoneal contents were washed with 170 ml chilled Hanks solution, which was subsequently filtered through several layers of cheese cloth. The cells were harvested by centrifugation at 700 rpm for 20 min in a swinging-bucket International centrifuge at 5°C. The pellet of cells obtained was resuspended with 0.5 ml Hanks solution. A differential cell count of the peritoneal exudate from animals of both strains, revealed that polymorphonuclear cells, monocytes and lymphocytes were present in about equal proportions. Samples were taken from the cell suspensions

and then spotted and dried on Whatmann No. 540 filter discs. The radioactivity present was determined in an Ansitron liquid scintillation spectrophotometer.

The results of the experiment are given in the Table. It can be seen from these data that the strains manifested no apparent difference in their ability to phagocytize ¹⁴C-DNP₁₂-PLL₃₉₈. That the radioactivity observed in these cell preparations represents phagocytosis and not simply adherence of antigen to cell surfaces is indicated by the fact that further washings of the cells did not diminish their radioactivity. The yield of cells from these guinea-pigs was low when compared with the yields obtained from rats 72 h following beef heart infusion broth i.p. and, in addition, the relative proportion of monocytic cells was less⁷. However, the efficiency of phagocytosis was observed to be about 1-2% of antigen administered and this

Extent of phagocytosis of ¹⁴C DNP₁₂-PLL₃₉₈ by peritoneal exudate cells from strain II and XIII guinea-pigs

Strain	Peritoneal No. cells	Exudate cpm	Uptake ¹⁴ C DNP-PLL (µg per 10 ⁷ cells)
II	0.28 × 10 ⁷	15,420	33.9
XIII	0.30 × 10 ⁷	16,900	35.1

4 h prior to sacrifice, 2.6 mg ¹⁴C DNP₁₂-PLL₃₉₈ (1.63 × 10⁶ cpm/mg) were injected i.p. into male guinea-pigs of strain II and XIII, 2 from each strain, that had been previously primed, 3 days before experimentation, by treatment with a saline-glycogen solution injected i.p.

¹ B. B. LEVINE and B. BENACERRAF, *Science* 147, 517 (1965).

² S. BEN-EFFRAIM, S. FUCHS and M. SELA, personal communication (1966).

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⁵ N. A. MITCHISON, Cold Spring Harbor Symp., 32, 431 (1967).

⁶ B. B. LEVINE and B. BENACERRAF, *J. exp. Med.* 120, 955 (1964).

⁷ S. M. GAEKWAR, B. DAVIS and A. SEHON, *Fedn. Proc.* 527, Abstr. 1508 (1967).

happens to be comparable to the efficiency reported by ASKONAS and RHODES⁸ for rat peritoneal exudate cells phagocytosing iodinated haemocyanin, 3 days following beef heart infusion broth i.p.

Had the unresponsiveness of strain XIII guinea-pigs to an antigen such as DNP-PLL been due to an inability to phagocytize it, a qualitative difference might have been expected in the cellular absorbance of ¹⁴C-DNP₁₂PLL₃₉₈. As this was not the case, the strain XIII block inhibiting the synthesis of anti DNP-PLL antibodies is evidently 'extra-phagocytic'. It is, of course, possible to reason that a difference has been obscured in the phagocytic activity of a small, but significant class of cells. With regard to this possibility, the results demonstrate that such a class of cells, if it existed, represents less than 10% of all phagocytic cells present. A group of cells constituting a significantly greater fraction than this, would have fallen within the range of detectability. Phagocytosis of DNP-PLL at similar rates by both strains, clearly does not ex-

clude the existence of a metabolic block in strain XIII macrophages that could inhibit subsequent processing of the antigen.

Résumé. Respectivement, les cochons d'Inde des races II et XIII immunisés répondent et ne répondent pas au poly-L-lysine conjugués. Par contre, nous savons qu'ils sont également capables de phagocyter le ¹⁴C-DNP-PLL dans la mesure où le sont les cellules des exudats du péritoine.

B. K. DAVIS

*Chemistry Department, McGill University,
Montreal 130 (Canada), 2 December 1969.*

⁸ B. A. ASKONAS and J. M. RHODES, Proc. Symp. *Molecular and Cellular Basis of Antibody Formation* (Ed. J. STERZL; Czech. Acad. Sci., Praha 1964), p. 508.

The Role of the Thymus in Antinuclear Autoimmunization

In earlier experiments¹⁻⁵ it was shown that thymectomy of the new-born mouse leads to the appearance of antinuclear antibodies in a high percentage of cases. Other authors have also noted the appearance of autoimmunization symptoms after thymectomy, in mouse and rabbit^{1,6-8}. To explain this autoimmunization two principal theories were advanced: (1) Thymectomy might be directly responsible for the formation of autoantibodies if it is recognized that one of the functions of this organ is inhibition of development of autoimmune reactions. (2) Auto-immunization might also be an indirect result of suppression of the thymus via the immune defect following thymectomy, which permits invasion of the organism by pathogens (viruses especially) that might be the essential cause of the signs of autoimmunization. To obtain arguments in favour of one of these theories, a study was made of the appearance of antinuclear factors in 29 CF1 inbred mice thymectomized at the age of 1 month. The antibodies were determined by the immunofluorescence reaction on blood smears from mice infected with trypanosoma gambiense according to usual technique⁹. The animals subjected to late thymectomy did not show any immune defect for several months, but from the third months onwards (Table I) antinuclear antibodies were seen to develop in a manner analogous to that seen in the CF1 mice thymectomized at birth. Nevertheless, the percentage of positive reactions in the animals thymectomized at the age of 1 month was lower than that found in the CF1 mice thymectomized at birth: 50% as against 71%. These findings appear to favour hypothesis (1), direct regulation of the autoimmunity by the thymus. The animals did not have the immune defect seen in the animals thymectomized at birth and invasion of a pathogen cannot be used to explain the autoimmunization. The smallest percentage of animals with autoantibodies in this experiment, compared with the animals thymectomized at birth, should be compared with the results obtained in the pathogen-free CF1 mice thymectomized at birth², where positive results amounted to 25% only. It seems, therefore, that although an infection is not necessary for the appearance of antinuclear antibodies, it may be an accessory favouring factor.

Again to permit a choice between the two principal hypotheses proposed, a study was made of the possibility of adoptive transfer of the antinuclear autoimmunity appearing in the animals thymectomized at birth. This was done in the following manner.

Table I. Antinuclear antibodies in CF1 mice thymectomized at the age of 1 month

Titres	Number of months after thymectomy						
	3	4	5	6	7	8	9
8	4	6	5	5	5	6	5
64	—	—	6	6	6	4	4
512	—	—	1	1	1	2	2
Total of mice with antinuclear antibodies	4	6	12	12	12	12	11
Total number of mice	29	27	26	25	25	24	22
% of animals with antinuclear antibodies	13.8	22.2	46.1	48	48	50	50

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³ J. THIVOLET and J. C. MONIER, Rev. fr. Etud. clin. biol. 14, 182 (1969).

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⁵ J. THIVOLET, J. C. MONIER, J. P. RUEL and M. H. RICHARD, Nature 214, 1134 (1967).

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⁹ J. THIVOLET, J. C. MONIER, F. LALAIN, M. H. RICHARD, Ann. Inst. Pasteur 109, 817 (1965).