by ST. PIERRE[7] and ST. PIERRE et al.[8] and STEFANI and MOORE[12] would result in a diminished survival time.

Evidence for a direct action of PHA on the leukemic cells is provided by the results obtained from the study in which the number of cells inoculated was varied. With the smallest inoculum (10^3 cells), PHA treatment had no effect on survival time but with 10^6 cells, PHA increased survival time by 44%. Studies to be reported at a later date have shown that L1210 leukemic cells are agglutinated in vivo by PHA. A proposed mechanism could be that a critical number of cells is needed within a certain volume before the agglutinating action of PHA is expressed. Agglutination would inhibit the uptake of the leukemic cells by the blood stream and lymphatics and reduce the number which eventually lodge within the filtering organs. Agglutination may also inhibit the metabolic activity of the leukemic cells resulting in a reduced mitotic level.

At the present time it is not clear whether PHA affects the animal or the leukemic cells but it is evident that leukemic animals treated with PHA survive longer than the untreated controls. The mechanism of this effect is being investigated with a combination of in vivo and in vitro studies[13].

Résumé. Des souris ayant subi un traitement journalier au PHA, entrepris 4 jours avant l'implantation de cellules leucémiques L1210 on survécu plus longtemps que celles qui furent traitées au sel. Le traitement journalier entrepris 24 h après l'inoculation de cellules leucémiques n'a pas eu d'effet sur la durée de survie. Par le traitement au PHA combiné à la 6-mercaptopurine, la durée de vie fut plus longue que par celui que l'on obtint avec d'autres agents employés isolément. Un mécanisme proposé pour l'effet antileucémique de PHA est discuté.

R. M. FOLK and R. L. ST. PIERRE

Battelle Memorial Institute, Columbus Laboratories, and Department of Anatomy, The Ohio State University, Columbus (Ohio 43201, USA), 29 October 1970.

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Active Immunotherapy of Mouse RC 19 and E ♀ K1 Leukaemias Applied After the Intravenous Transplantation of the Tumour Cells[1]

In a series of experiments carried out on L 1210 leukaemia grafted subcutaneously, we showed that active immunotherapy can be effective, delaying and reducing the mortality, even when it is applied after the graft of the tumor cells[2,3]; the necessary condition of its efficiency was that the number of tumor cells be $\leq 10^5$.

In these experiments, the living tumour cells were grafted subcutaneously in such a way that the tumour volume could be measured and the effect of the immunotherapy on the tumour growth could be quantitatively estimated. Nevertheless, it is questionable whether the effect of active immunotherapy might not be due to the stimulation of a lymph node not yet immunologically informed at the time of the immunological stimulation by the tumour associated antigen, and if the result obtained could be found again in human leukaemia, which is, at least at the time of treatment, a disseminated neoplasia. Another criticism which could be made of our L 1210 leukaemia experiments concerns the history of this grafted tumour which has been transmitted by transplantation through many generations, and of which the tumour associated antigens are difficult to demonstrate[4].

Hence the idea of treating with active immunotherapy other experimental mouse leukaemias, the cells of which are 1. carrying tumour associated antigens which are well precized; 2. disseminated because inoculated intravenously.

Material and methods. 1. *Leukaemia RC 19.* 60 (DBA/2 × Balb/c) F1 female mice, aged 3 months, received by intravenous route 10^3 cells from (Rauscher) leukaemia RC 19, which is transmissible by graft. These animals were divided at random into 4 groups: group a) comprised 15 controls; group b) 15 mice which received 24 h after the leukaemia graft 1 mg living B.C.G.[5] intraperitoneally, this injection being repeated every 4 days for 16 days; group c) comprised 15 mice which received 48 h after the graft of the leukaemia a peritoneal injection of 10^7 formalized isogenic leukaemic cells, the injection being repeated every 4 days for 16 days; group d) comprised 15 mice which received the combination of both treatments.

2. *Leukaemia E ♀ K1.* 75 C_{57}Bl/6♀ mice, aged 2 months, received by intravenous route 10^3 cells from E ♀ K1

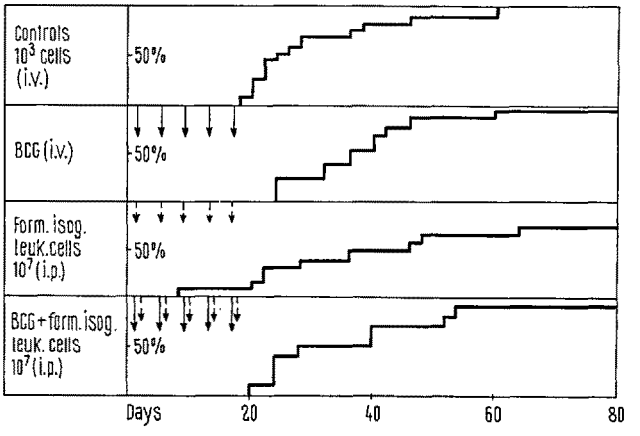

Fig. 1. Cumulative survival of mice carrying E ♀ K1 leukaemia not treated, or treated by B.C.G., or formolinized isogenic leukaemic cells, or by combination of both.

[1] This work has been carried out with the aid of INSERM, contract No. 66–235.

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[5] From Institut Pasteur, Paris.

leukaemia, which is a grafted leukaemia arizing from a leukaemia induced in $C_{57}Bl/6$ mice by the Gross virus. These animals were divided at random into 4 groups: group a) comprised 15 controls; group b) 12 mice which received 24 h after the graft of the leukaemia 1 mg living B.C.G. i.p.; this injection being repeated every 4 days for 16 days; group c) comprised 12 mice which received 48 h after the graft of the leukaemia a peritoneal injection of formalized 10^7 isogenic leukaemic cells; this injection being repeated every 4 days for 16 days; group d) comprised 12 mice which received the combination of both treatments.

Results and discussion. Figure 1 shows the results obtained in E ♀ K1 leukaemia: B.C.G. given alone had no significant action; formolinized isogenic leukaemia cells given alone have a very powerful action; the association of both had a weak, but statistically significant effect (S to 4%).

Figure 2 shows the results obtained in RC 19 leukaemia: the effect of B.C.G. given alone is considerable (S to 1 p. 1000); the effect of formolinized tumour cells is very weak, but the effect of the combination of both is similar to the effect of B.C.G. given alone (S to 1 p. 1000).

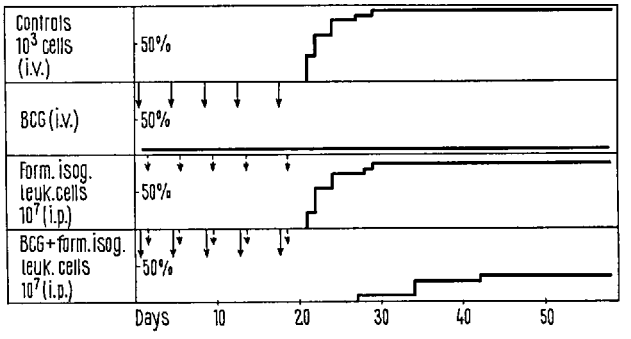

Fig. 2. Cumulative survival of mice carrying RC 19 leukaemia, not treated, or treated by B.C.G., or formolinized isogenic leukaemic cells, or by combination of both.

In conclusion, active immunotherapy applied after the intravenous graft of tumour cells from 2 leukaemias originally induced by virus and of which the tumour associated antigens are well known, can be active, as it has been shown active previously by us on experiments with L 1210 leukaemia, which justified a clinical trial that we have conducted on acute lymphoblastic leukaemia in man, the results of which are very encouraging[6,7].

Résumé. L'immunothérapie active non spécifique ou mixte, appliquée 24 h après la greffe isogénique de la leucémie RC 19, possède une action considérable; appliquée dans les mêmes conditions après la greffe de la leucémie E ♀ K1, elle possède une action modérée mais significative. Ces résultats confirment l'effet antileucémique de l'immunothérapie active appliquée après le début de la maladie et montre qu'elle est même efficace sur les cellules tumorales disséminées.

G. Mathé, P. Pouillart
and Françoise Lapeyraque

Institut de Cancérologie et d'Immunogénétique, Hôpital Paul-Brousse, 14, Avenue P. V. Couturier, F-94 Villejuif (France), 28 September 1970.

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The Chemical Nature of the Pig Blood-Group Substances Dissolved in the Serum

It is now well established that the determinants (haptenic sites) of the classical human blood-group substances are sequences of various sugars and/or N-acetylhexosamines[1,2]. There is a possible diversity of macromolecules carrying identical determinants. Thus, the cellular ABH-substances of man seem to be bound to lipids[3], the soluble ABH-substances present in the secretions of about 80% of individuals, however, are bound to glycoproteins[1]. The Lewis antigens in human erythrocytes are also bound to lipids[4], while the Lewis substances present in secretions occur in the form of glycoproteins. Some peculiar blood-group substances are also found dissolved in the serum, e.g. the Lewis antigens of man[5], the J antigen of cattle[6], the A antigen of pig[7,8] and the Na and Nb antigens of pig[9]. Since it has been demonstrated that the bovine J antigen in the serum[10] and recently also the human Lewis antigen in the serum[11] are bound to lipids, it appears reasonable to extend similar investigations to the other above-mentioned blood-group substances dissolved in sera.

With respect to the occurrence of dissolved antigens, the pig sera used were termed A^-Na/a, A^-Nb/b, A^+Na/b and A^+Nb/b. In one experimental series the total lipids were extracted with ethanol/diethyl ether (3:1, v/v)[12] and purified by the Folch procedure[13], in another experimental series they were extracted with methanol/chloroform (1:1, v/v) according to the description given[14] for the extraction of red cells and subsequently purified by passing the lipid solution through Sephadex G-25 fine[15]. The mucoproteins were extracted with cold phenol/water (3:1, w/w)[16] and purified by precipitation with ethanol containing potassium acetate[17]. The yield of total lipids was about 180–320 mg/100 ml serum, the yield of mucoproteins was about 15–20 mg/100 ml serum.

The total lipids were emulsified in isotonic (0.15*M*) saline by use of glass homogenizers giving a concentration of 10 mg lipid/ml. The mucoproteins were readily soluble in isotonic saline; a solution of 5 mg/ml was prepared. The immunological activities of those preparations were tested by their abilities to inhibit the specific antibody-antigen-reactions. The inhibition tests with the incomplete anti-Na and anti-Nb sera were carried out as described earlier[9]. The activities in the A system were measured by using hemolysis-inhibition tests. The titers of the 3 antisera used were $^1/_4$ each.

We found (Table) Na and Nb activities in the corresponding mucoproteins only, while both total lipids and mucoproteins extracted from A pig serum showed specific activities. These results indicate that the Na and Nb determinants are bound to porcine serum muco-