INDUCTION OF RESISTANCE AGAINST ISOTRANSPLANTATION
OF VIRUS-INDUCED MYELOID LEUKAEMIAS

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ALL the experiments carried out in various laboratories since the publications of Foley (1953) and Prehn and Main (1957) concerning the antigenicity of experimental tumours confirmed that sarcomas induced by carcinogenic hydrocarbons contain specific antigens (Révész, 1960 ; Klein *et al.*, 1960 ; Koldovský, 1961*a* ; Old *et al.*, 1961 ; Pasternak, Horn and Graffi, 1962*a*). This conclusion, for example, resulted from experiments in which resistance against cell inocula of the same isologous tumour could be produced by pretreatment of mice with X-ray irradiated tissue suspensions of a tumour or after ligation and following regression of a subcutaneous or an intradermal tumour graft.

Since immunologic cross-reactions, even between tumours which were induced by the same carcinogen, could not be observed by most of the investigators (Klein *et al.*, 1960 ; Prehn, 1961 ; Feldman, Globerson and Yaffee, 1962 ; Pasternak *et al.*, 1962*b*, in contrast to Koldovský, 1961*b*) for each of these tumours, individually specific antigens may be suggested.

A specific antigenicity could also be demonstrated for the tumours in mice induced by the polyoma virus. In contrast to the carcinogen-induced tumours at least a great number of those tumours in mice induced by this virus, seem to contain the same cellular antigen (universal antigen) (Habel, 1961 ; Sjögren, 1961), since resistance against isologous tumour grafts developed both after treatment of the animals with the virus and after homotransplantation of the polyoma tumours. Virus-neutralizing antibodies did not affect the growth of the tumours.

These differences concerning the antigenic properties of tumours of different aetiology suggested an investigation of the antigenicity of virus-induced leukaemias.

The results of earlier experiments (Pasternak *et al.*, 1962*c*) already indicated the possibility of an active immunization at least against one of the two transplantable myeloid leukaemias of the strains C57Bl and CBA tested. Furthermore, Klein, Sjögren and Klein (1962) were able to prove in extensive experiments that also in mice a state of relative resistance may be produced against lymphomas, induced by the Gross agent, if the recipients were pretreated with homografts of these leukaemias or with sub-threshold cell doses of the same isologous lymphoma. The partial or complete cross-reactions obtained for the different lymphomas, indicate that similar or identical antigens may be found in these virus tumours which are however different from those of polyoma tumours.

In the present paper the existence of specific antigens in isologous grafts of the virus-induced myeloid leukaemia of mice (Graffi *et al.*, 1954) will be described.

Since in previous experiments (Pasternak and Graffi, 1961) common antigenic components in viruses of the myeloid and the lymphatic leukaemia could be proved, we were especially interested in the antigenic relations between the different types of leukaemias induced by these viruses.

MATERIALS AND METHODS

Experimental animals and tumour donors were inbred mice of the strains CBA, C57Bl, XVII and AKR. In one of the experiments F_1 -hybrids produced by crossing XVII females and CBA males were used. All animals were taken from a breed which is maintained by brother-to-sister mating (Pasternak and Grysczek, 1962). The skin graftings performed showed the homogeneity of the strains used; the survival of the grafts exceeded 150 days.

The leukaemias used for the experiments and their data are presented in Table I. All of the myeloid leukaemias were induced by injection of cell-free filtrates of

TABLE I.—*Some Data on the Leukaemias used for the Experiments*

Designation	Induced by	Latency period (days)	Strain of origin	Number of transfer generations in isologous hosts	Histological type
PL 1462	Virus of myeloid leukaemia	161	CBA	46	Myeloid leukaemia
L 129/6d	"	158	C57Bl	17	"
					(Chloroleukaemia)
L 112b	"	206	C57Bl	21	Myeloid leukaemia
L 184/3a	"	119	XVII	14	"
L 1205	Gross agent (spontaneously developed)	303	AKR	2	Lymphatic leukaemia
L 1061	"	326	AKR	—	"
L 1937	"	216	AKR	—	"

virus-induced leukaemias in newborn mice. These leukaemias were transplanted to the original strain. In these experiments each of the leukaemias was tested in different generations of transplanting, in order to determine possible changes in the antigenic composition. The lymphatic leukaemias developed spontaneously in mice of the AKR strain containing the Gross agent. Lymph nodes and spleens from animals with leukaemias L 1061 and L 1937 were transplanted for immunization purposes exclusively to homologous recipients; in addition the leukaemia L 1205 was further transplanted to isologous recipients.

In each of the transplantation or immunization experiments performed with leukaemic materials the sex of the animals was taken into consideration to exclude reactions against Y-linked H-antigens. Leukaemias however which originated from females were also transplanted to males. All experimental animals were of uniform weight; their age ranging from 3 to 4 months. For purposes of immunization the animals were treated as follows:

(1) At intervals of two weeks isologous recipients received 2 to 3 subcutaneous injections of a suspension from leukaemic tissue (0.2 to 0.3 ml. each), which had been irradiated *in vitro* at a dose of 14500 r. For that purpose the tissue (intramuscular graft, lymph nodes or spleens) was cut into small pieces with scissors and

slightly homogenized by hand in a small quantity of a phosphate-buffered isotonic saline solution using a Potter glass homogenizer. Previous to irradiation the suspension which contained for the most part intact cells and cell aggregates was further diluted with a saline solution to a ratio of 1 : 4 as compared to the initial weight of the material.

(2) Under similar experimental conditions homologous recipients received subcutaneous injections of the same but non-irradiated tissue suspension in doses of 0.2 to 0.3 ml. In general one single treatment was sufficient to produce resistance against isologous grafts of myeloid leukaemia.

(3) Isologous recipients were treated by subcutaneous injections of sub-threshold cell doses (in some cases up to 5×10^4 cells). Only those animals were taken for the tests which did not show any subcutaneous tumours or in which the tumours regressed after temporary growth.

Test injections for the determination of the induced resistance were made either 1-7 days after treatment with the irradiated tissue suspensions or at particular intervals after the animals had been pretreated with sub-threshold cell doses. The cell suspensions were obtained by trypsination (0.15 per cent solution) of the tissue material (Graffi, Pasternak and Horn, 1962). After vital staining with trypan blue the cells were counted and adjusted to the doses required.

The animals were pretreated by left lateral subcutaneous injections while the test injections were given subcutaneously on the right side. In some experiments the intravenous mode of administration was chosen. The results of experiments presented in the different tables include the number of resistant animals in comparison with the total number of test animals which were fit for evaluation.

RESULTS

Induction of resistance against grafts of virus-induced myeloid leukaemias by pre-treatment of mice with isologous tumour material

The results summarized in Table II indicate that 78.9 per cent of the pre-treated animals are resistant to cell inocula of isologous leukaemias as compared with 12.0 per cent of the untreated controls. This difference is statistically highly significant and implies that the pretreatment gave rise to an active defence of the host. The effect was marked in all three strains of mice, on each of which one type of myeloid leukaemia was tested. It should be mentioned, however, that the method employed for immunization plays an essential role for producing this resistance. After treatment of CBA mice with irradiated tissue of leukaemia PL 1462 it was at no time possible to demonstrate a resistance against grafts of this isologous tumour, while mice of the strain XVII revealed a strong resistance having received the same treatment with tissue material of leukaemia L 184/3a—both after subcutaneous and intravenous test injections. That the leukaemia PL 1462 contains specific antigens and that CBA mice were able to react against this type of leukaemia results from the fact that after pretreating the animals with sub-threshold doses of cells, the number of takes in the test group was noticeably diminished in comparison with those in the control group.

The results obtained after immunization of C57Bl mice with tissue material from the leukaemia L 129/6d indicate that an active resistance was produced by using both methods of immunization. Detailed data on the varying effectiveness of certain methods of immunization will be published later.

TABLE II.—*Results of the Experiments on the Induction of Resistance Against Grafts of Virus-induced Myeloid Leukaemias by Pretreatment of Mice with Isologous Tumour Material*

Mouse strain inoculated	Line of leukaemia used for pretreatment	Genotype of origin	Pretreatment	Designation of challenge leukaemia	Number of cells inoculated	Route of administration	Immunized group	Control group
CBA	PL 1462	(CBA)	Injection of small doses of viable cells	PL 1462 PL 1462	5×10^4 5×10^4	i.v. i.v.	41/46* 18/19	4/9* 4/9
C57Bl	L 129/6d	(C57Bl)	Injection of irradiated tissue suspension	L 129/6d	5×10^4 10^5	s.c. s.c.	7/18 8/19	1/9 0/10
			Injection of small doses of viable cells		10^5 5×10^4	i.v. i.v.	15/22 20/22	0/10 0/9
XVII	L 184/3a	(XVII)	Injection of irradiated tissue suspension	L 184/3a	5×10^4 5×10^4	s.c. i.v.	19/19 10/10	0/9 0/10
Total :							138/175	9/75
Percentage of resistant animals :							78.9	12.0
							χ^2	9.4
							P	<10 ⁻¹⁰

* Number of resistant animals over the total number inoculated.

Induction of resistance against isologous grafts of virus-induced myeloid leukaemias by pretreatment of mice with homologous grafts of myeloid leukaemias (immunologic cross-experiments)

Inbred mice, in which homologous grafts of virus-induced myeloid leukaemias have regressed, reveal a relative resistance to isologous grafts from leukaemias of the same origin. This conclusion can be drawn from the results of experiments presented in Table III. In addition the table contains the results of cross-experiments carried out with leukaemias L 129/6d and L 112b on C57Bl mice. C57Bl mice in which resistance was produced against cell inocula of the leukaemia L 129/6d were also resistant to isologous grafts of the leukaemia L 112b. The final results of 63.7 per cent of resistant animals in the test group as compared with 33.3 per cent in the control group is statistically highly significant.

Experiments to produce resistance against isologous grafts of virus-induced myeloid leukaemias by pretreatment of mice with homologous grafts of virus-conditioned lymphatic leukaemias

The three experiments hitherto performed utilized different lines of the lymphatic leukaemia for the pretreatment of mice of the strains XVII and C57Bl. They showed the progressive growth of isologous grafts of myeloid leukaemias on these animals. In comparison with the controls even a certain growth-stimulating effect was found in mice subjected to treatment. While homologous grafts of myeloid leukaemias induce an active resistance against cell inocula of isologous leukaemias having the same origin, homologous grafts of spontaneous virus-induced leukaemias of the AKR strain are not able to produce resistance against grafts of myeloid leukaemias. The percentage of resistant animals in the test group was 1.9 as against 7.1 in the control group.

TABLE IV.—*Results of the Experiments to Produce Resistance Against Isologous Grafts of Virus-induced Myeloid Leukaemias by Pretreatment of Mice with Homologous Grafts of Virus-conditioned Lymphatic Leukaemias*

Mouse strain inoculated	Line of leukaemia used for pretreatment	(Genotype of origin)	Designation of challenge leukaemia	Number of cells inoculated	Immunized group	Control group
XVII	L 1205	(AKR)	L 184/3a	5×10^4	0/19*	0/10*
C57BL	L 1061	(AKR)	L 129/6d	10^5	1/20	2/9
C57BL	L 1937	(AKR)	L 129/6d	3×10^5	0/13	0/9
Total :					1/52	2/28
Percentage of resistant animals :					1,9	7,1
χ^2						1,5
P						>0,3

* Number of resistant animals over the total number inoculated.

DISCUSSION

The experiments performed indicate the possibility of producing resistance against isologous grafts of virus-induced myeloid leukaemias by pretreatment of mice with cells or tissues of transplantable leukaemias of the same origin. Such a resistance is ascertainable if about 10^4 to 10^5 cells are given subcutaneously or intravenously; the percentage of resistant animals in the test group is significantly

TABLE III.—*Results of the Experiments on the Induction of Resistance Against Isologous Grafts of Virus-induced Myeloid Leukaemias by Pretreatment of Mice with Homologous Grafts of Myeloid Leukaemias*

Mouse strain inoculated (XVII × CBA)F ₁	Line of leukaemia used for pretreatment	Genotype of origin	Designation of challenge leukaemia	Number of cells inoculated	Route of administration	Immunized group	Control group
(XVII × CBA)F ₁	L 184/3a*	(XVII) (C57Bl)	PL 1462	10 ⁵	s.c.	10/10†	6/10†
	L 112b	(C57Bl)		3 × 10 ⁵	s.c.	19/19	6/10
CBA	L 129/6d	(C57Bl)		5 × 10 ⁴	s.c.	12/18	13/20
	L 129/6d	(C57Bl)		5 × 10 ⁴	i.v.	16/16	6/9
C57Bl	PL 1462	(CBA)	L 129/6d	5 × 10 ⁴	s.c.	15/20	12/19
	PL 1462	(CBA)		5 × 10 ⁴	s.c.	3/10	0/10
C57Bl	L 184/3a	(XVII)		5 × 10 ⁴	s.c.	7/13	1/13
	L 184/3a	(XVII)	L 112b	5 × 10 ⁴	s.c.	11/20	4/10
XVII	L 129/6d†	(C57Bl)		10 ⁵	i.v.	11/16	5/17
	PL 1462	(CBA)	L 184/3a	2 × 10 ⁵	s.c.	3/9	0/20
	PL 1462	(CBA)		10 ⁵	s.c.	9/20	0/10
	L 129/6d	(C57Bl)		10 ⁵	s.c.	5/19	0/11
Total :				121/190		53/159	33,3
Percentage of resistant animals :				63,7		33,7	
					χ^2		
					P		
							<10 ⁻⁸

* Tissue suspension irradiated with 14500 r *in vitro* before inoculation.

† Number of resistant animals over the total number inoculated.

‡ Recipients immunized by injection of small doses of viable L 129/6d-cells.

higher than in the group of the controls which had not been immunized. The treatment of control animals with normal tissues was considered dispensable since previous experiments (Pasternak *et al.*, 1962a) had proved that the growth of isologous tumour grafts was not influenced by this pretreatment.

Considering these results obtained with myeloid leukameias and the findings of Klein *et al.* (1962) resulting from work with lymphatic leukaemias and taking into consideration studies by Sachs (1962), it may be assumed that in general virus-induced leukaemias in mice reveal a specific antigenicity.

In the experiments of Sachs, who tested leukaemias induced by the Moloney virus, a resistance against isologous grafts of these leukaemias was also produced by pretreatment of mice with a virus-containing tissue culture fluid. This would be in approximate accordance with the effect observed by Habel (1961) and Sjögren (1961) for the polyoma virus which showed that the growth of polyoma tumour grafts is inhibited if adult mice had been pretreated with this virus. Klein *et al.* (1962) were not able to detect cross-reactions between the leukaemias and the polyoma tumours, a fact indicating the existence of different antigens. Yet, the mode of induction of a new cellular antigen by the action of either a DNA-virus or a RNA-virus seems to be most similar. The present results do not allow assumptions as to whether the specific antigenicity is confined exclusively to the malignant cell. And, similarly, the role which viral antigens play in producing a resistance to isologous grafts of leukaemias is not thoroughly clear. In a previous paper (Pasternak and Graffi, 1961) we were able to show that the virus of myeloid leukaemia is inactivated both by antisera against cell-free filtrates of myeloid leukaemias and by similar antisera against lymphatic leukaemias (induced by the Gross agent). These viruses seem to contain common antigens though the cellular antigens of both types of leukaemia are probably different as can be seen from the absence of cross-reactions described for the present experiments. It remains to be seen which antigenic correlations will be found to exist between other types of leukaemias induced by different viruses and those already tested.

The absence of cross-reactions between virus-induced lymphatic leukaemias and leukaemias of different aetiology (Slettenmark and Klein, 1962) is a proof of the exceptional position virus-induced leukaemias occupy. Judging from the results hitherto available the existence of a universal antigen of leukaemias appears to be doubtful.

SUMMARY

Resistance against isotransplantation of virus-induced myeloid leukaemias of mice was produced by pretreatment of the animals with irradiated tissue suspensions of these leukaemias or by inoculation of small doses of viable cells prepared from isologous leukaemic material or by homotransplantation of leukaemic tissue of the same origin. Mice treated under equal experimental conditions with homologous grafts of spontaneous lymphatic leukaemias of the AKR strain did not develop any resistance against isotransplantation of myeloid leukaemias. The conclusion drawn is that the cells of the two types of leukaemias contain different antigens.

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