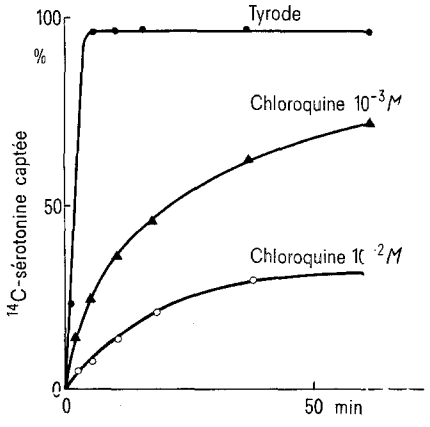

Fig. 1. Effet de la chloroquine sur la captation de ^{14}C-sérotonine. Chaque point représente la moyenne de 2 expériences.

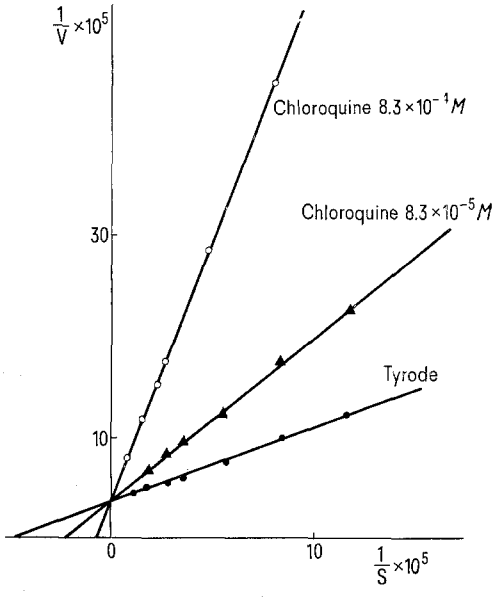

Fig. 2. Représentation selon LINEWEAVER et BURK de la captation de ^{14}C-sérotonine. Chaque point représente la moyenne de 2 expériences.

La chloroquine ne modifie ni le nombre des granules plaquettaires ni leur volume (Tableau II). Ce résultat prouve aussi que la ^{14}C-sérotonine libérée est d'origine cytoplasmique et non granulaire. Ainsi, la chloroquine a une action différente sur les granules plaquettaires et leucocytaires puisqu'elle provoque une vacuolisation de ces derniers avec formation de figures myéliniques[3-5]. Par contre, dans les plaquettes, elle modifie la membrane plasmique en dilatant le système des vacuoles communiquant avec la surface (SCS) qui correspond à une invagination de la membrane plaquettaire.

Les mesures de captation de sérotonine mettent en évidence une inhibition de ce phénomène (Figure 1) suggérant aussi une altération de la membrane; le diagramme de LINEWEAVER et BURK montre en effet que l'inhibition est compétitive (Figure 2).

Ainsi l'ensemble de ces résultats démontrent que la libération de sérotonine observée est bien d'origine cytoplasmique et qu'elle est la conséquence d'une perméabilité passive puisque la captation active est inhibée. Ceci est corroboré par le fait qu'en présence de sérotonine froide extraplaquettaire (2×10^{-3} *M*) la libération de ^{14}C-sérotonine sous l'action de la chloroquine est très diminuée (Tableau I).

L'inhibition de la captation est de type compétitif et pourrait résulter d'une interaction de la chloroquine avec les D-récepteurs de la sérotonine[11].

Summary. On electron microscopy, platelets exposed to chloroquine exhibit no abnormality, except for surface connecting system. However, when platelets were labelled with ^{14}C serotonin, there was liberation of a large amount of radioactivity which is inhibited when the extracellular concentration of serotonin is increased. It appears that chloroquine is a competitive inhibitor of serotonin uptake by platelets.

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Cell-Mediated Immunity Associated with Long Term Transplantation Resistance to a Syngeneic Tumour of Spontaneous Origin. Detection by Two in vitro Tests

Transplantation resistance to syngeneic tumour cells has been shown to be accompanied by cell-mediated immunity detectable by in vitro tests[1-4]. It has been shown previously that a transplanted immunoglobulin-secreting sarcoma of spontaneous origin ($ISIS_{130}$) regressed in syngeneic rats after a single dose of cyclo-

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Table I. Percent migration of spleen cells in the presence of tumour cells

Tumour cell / Spleen cell ratio	Control rats			Resistant rats				Significance[a] (p)
1:20	80.67[b]		89.27	85.2	8.8	2.6	44.3	0.069
	84.84		54.57	189.6	42.9			
	98.56	98.20	65.16	91.2	46.53	146.04	44.81	
	76.33		49.19	45.41	31.70	48.73	59.38	
	100.28		95.45	37.76	112.92	90.64	142.19	
	mean = 81.13			mean = 70.59				
1:30	85.50		60.72	56.9	72.3	28.7	79.0	0.24
	41.17		44.98	128.4	11.9			
	93.89	66.20	78.44	100.7	43.37	106.43	79.11	
	86.90		35.94	53.66	39.89	72.87	51.87	
	79.22		81.06	85.61	112.94	90.78	131.27	
	mean = 68.54			mean = 74.76				
1:70	134.11		134.99	60.0	96.1	8.3	24.4	0.0023
	100.19		163.04	151.5	90.2			
	89.97	116.35	60.65	106.39	40.7	N.T.	67.8	
	189.56		171.88	82.06	40.94	90.32	64.70	
	95.66		105.82	68.52	94.55	89.74	126.63	
	mean = 123.83			mean = 76.63				
Overall mean		91.17				73.95		0.029

[a] Significance was assessed by Mann-Whitney U-test to give the probability that resistant rats are not different from control rats. [b] Each line represents 1 experiment within 1 tumour cell/spleen cells ratio. Corresponding lines with other ratios are derived from the same experiments. N.T., not tested.

Table II. Percent inhibition of migration in rats resistant to $ISIS_{130}$

Experiment	Rats	Tumour cell/spleen cell ratio			Average
		1:20	1:30	1:70	
1	1	0	22.1	55.4	25.82
	2	89.6	1.1	28.6	39.78
	3	96.9	60.7	93.8	83.80
	4	48.0	— 8.1	81.8	40.58
2	1	—172.0	—196.0	—15.1	—127.73
	2	38.4	72.6	31.5	47.49
3	1	— 4.4	— 26.6	—19.5	— 16.85
	2	46.7	40.4	54.2	47.13
	3	— 67.2	— 33.8	N.T.	— 50.53
	4	48.7	0.6	23.8	24.32
4	1	27.6	12.6	54.6	31.62
	2	49.5	35.0	76.9	53.80
	3	22.3	— 18.7	50.0	17.90
	4	5.4	15.5	64.2	28.36
5	1	61.4	— 6.8	31.9	28.85
	2	7.4	— 13.3	10.9	1.69
	3	— 15.4	— 40.9	6.2	— 16.70
	4	— 45.3	— 63.8	—25.7	— 44.92

N.T., not tested.

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phosphamide. The treated rats subsequently developped a long-lasting specific resistance to a challenge with the same tumour cells[5]. It is anticipated that cell-mediated immune mechanisms are involved in this resistance. In the present experiments, two tests were used to demonstrate cell-mediated immunity in vitro in rats which display long-term resistance to $ISIS_{130}$, and to assess further the antigenicity of this tumor of spontaneous origin.

Materials and methods. LOU/C rats were made resistant to $ISIS_{130}$ and challenged as previously described[5]. 7 to 13 months after the first graft, a last challenge of 10^6 $ISIS_{130}$ cells was given, 2–4 days before each in vitro test. $ISIS_{130}$ grown in ascites form were used as target cells. Effector cells were obtained by dissociation of spleen cells. Direct MIF tests were performed according to Lespinats and Poupon[6]. The percent migration[7] and the percent inhibition of migration for resistant rats[8] were calculated as described. Cytotoxicity tests were performed as described by Brunner et al.[9]. However the cells were incubated 20–24 h. In each test, 2 or 3 control rats of the same age were processed along with the resistant rats.

Results. As seen in Table I, migration was reduced in the presence of $ISIS_{130}$ cells both for normal spleen cells and for spleen cells from resistant rats at tumour cell to spleen cell ratios of 1:20 and 1:30 but only with the latter cells at the ratio of 1:70. The overall difference between percent migration of control and resistant rat spleen cells is significant (p 0.029) but more so at the ratio 1:70 (p 0.0023). The percent inhibitions of migration (Table II) were positive for 13/18 rats, most of them in the range of 25 to 55. Most animals had either positive or negative values at all 3 ratios of tumour to spleen cells. Chromium-release tests for cytotoxicity of spleen cells

Table III. Results of cytotoxicity test in rats resistant to $ISIS_{130}$

Experiment	Spleen cell / Tumour cell ratio	Rat	cpm ± S.E.[a]	C.I. (%)[b]	P (2 α)[c]
1	300:1	1	1174 ± 23	15.76	< 0.001
		2	1169 ± 18	15.31	< 0.001
2	300:1	1	3228 ± 36	7.67	< 0.02
		2	3208 ± 37	8.73	< 0.025
3	300:1	1	1108 ± 58	−0.3	N.S.
4	300:1	1	1414 ± 44	−10.8	< 0.025
		2	1577 ± 29	− 0.9	N.S.
5	50:1	1	696 ± 20	5.0	N.T.
6	300:1	1	2973 ± 63	68.9	< 0.001
		2	2592 ± 49	35.5	< 0.001
		3	2385 ± 34	17.4	< 0.001
7	150:1	1	1120 ± 13	24.7	< 0.001
		2	1104 ± 15	22.1	< 0.001
8	300:1	1	2740 ± 76	0.67	N.S.
		2	3095 ± 63	18.8	< 0.001
		3	3054 ± 64	16.7	< 0.001
		4	2755 ± 39	1.4	N.S.

[a] Standard error of the mean. [b] Cytotoxicity index. [c] The significance of the difference from the control rats was determined by Student's t-test. N.S., non significant.

demonstrated significant cytotoxicity in 11/17 resistant rats. Again individual differences were observed among individual rats, although the internal variations within each rat were small (SE: 2–5% of the mean).

Discussion. Our results show that the persistance of transplantation resistance is accompanied by a demonstrable cell-mediated immunity in the majority of the rats. This immunity was detected in vitro as late as $7\frac{1}{2}$ to 13 months after the primary sensitization. Migration inhibition tests showed that, in the majority of resistant animals, spleen cells produced a stronger inhibition of migration in the presence of tumour cells than did control spleen cells. Cytotoxicity tests gave results along the same lines. The majority of the resistant rats had a significant cytotoxicity index compared to control rats whereas a few rats although resistant to tumour challenges did not show in vitro cytotoxicity of their spleen cells.

The discrepancy between the 2 in vitro tests and transplantation experiments leads to several considerations. First, the in vitro tests used may be less sensitive to detect tumour immunity than the transplantation experiments, possibly because immune cells may not be favourably concentrated in the spleen. Second, spleen cells may provide inhibitory activity for the reactivity of the specifically sensitized cells possibly through blocking factors[10]. Third, in vivo and in vitro experiments may detect different antigens or different sets of antigens. At present, no conclusion can be drawn here.

In most migration inhibition tests it was observed that the tumour cells produced an effect on the migration of control spleen cells and that this effect was dose-dependent. This could represent toxic phenomena or a lower reactivity of control spleen cells of an immune nature.

In conclusion, it has been shown that transplantation resistance to a syngeneic tumour of spontaneous origin is accompanied by cell-mediated immunity to the tumour cells detectable by two in vitro tests several months after the primary immunization. These results further substantiate the presence of tumour associated antigens in these tumour cells.

Résumé. Deux tests in vitro ont permis de détecter une immunité cellulaire antitumorale chez des rats résistant à la transplantation d'un sarcome d'origine spontanée ($ISIS_{130}$). Ces résultats sont une indication supplémentaire de la présence d'antigènes associés aux tumeurs sur ces cellules.

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