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THE PRESENCE OF MOLONEY VIRUS INDUCED ANTIGEN ON ANTIBODY-PRODUCING CELLS

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SUMMARY

Mice infected at birth with Moloney virus were stimulated with an unrelated antigen (sheep red blood cells) during the lag period before the outbreak of leukaemia. Their spleen cell suspensions were incubated with anti-Moloney serum and complement before plating by the Jerne technique. This treatment—compared with incubation with normal serum and complement—caused a decrease in number of plaque forming cells in one-fourth of the infected mice, indicating that their antibody producing cells contained virus specific surface antigen.

INTRODUCTION

In the process of viral oncogenesis there are at least two operationally distinct phenomena: (a) the loss of normal growth and differentiation patterns of the target cell, and (b) the appearance of virus-induced group specific membrane antigen in the transformed cells (Klein, Klein & Haughton, 1966).

The relation between these two events is not known, the extreme possibilities being: (1) the appearance of antigen is a secondary consequence of viral oncogenesis, and (2) the appearance of a new antigen on the membrane is responsible for the altered reactivity of the cell to the environmental signals that govern ordinary tissue differentiation and maturation.

The present study is an attempt to decide between these two alternatives, by testing whether a *functionally intact* normal cell can carry a virus induced antigen on its surface, as is suggested by some data in the literature (Breyere & Williams, 1964; Stück, Old & Boyce, 1964; Svet-Moldavsky, Mkheidze & Liozner, 1967; Pasternak, 1969; Mathé, 1969).

It was important to set the functional criteria within a histogenetic context that is pertinent to the target cells of the actual neoplastic transformation. We therefore chose a lymphoma-inducing agent (MLV, or Moloney virus), and the performance of a specific immune response—a process known to involve a series of mitoses, cell maturation and synthesis of

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a specific immunoglobulin—as the functional criterion. We used the Jerne technique (Jerne, Nordin & Henry, 1963), permitting the enumeration of plaque forming cells (PFC) in a splenic population, by virtue of their ability to produce zonal haemolysis in agar blood. The same cells were also tested for presence of the Moloney antigen, by their cytotoxic susceptibility to pretreatment with anti-Moloney serum and complement judged by a decrease in PFC, as previously described for *H*–2 antigens (Celada & Klein, 1967).

MATERIALS AND METHODS

Animals

Mice of the inbred strains A/Sn, C57 leaden and C57BL, and F_1 hybrids of the types ABY × DBA/2, A/Sn × DBA/2, C3H × C57BL and A/Sn × C57 leaden were injected with Moloney virus (MLV) at birth. All mice used in the experiments had no cytotoxic anti-MLV antibodies in their serum, and were by this criterion considered 'tolerant'.

Sera

Anti-Moloney sera were produced by inoculating adult (ABY × DBA/2) F_1 mice with 5×10^6, X-irradiated (6000 r) cells of the syngeneic YDYA Moloney-induced lymphoma of (ABY × DBA/2) F_1 origin. They had cytotoxic indexes between 0·70 and 0·98 against standard YAC target cells. Taking an index of 0·30 as the endpoint, cytotoxic titres varied between 1:8 and 1:32.

Experimental design

Each experiment was performed in the following way: a group of two to four MLV-injected mice and a similar number of controls of the same age, sex and strain received one injection of 4×10^8 sheep red blood cells (SRBC) intravenously at the age of 2–3 months. They were killed 5 days later and half of their spleen was teased in 1 ml of Eagle's medium. From each cell suspension, volumes of 0·03 ml were transferred to five or six small tubes; to two of these tubes 0·02 ml of undiluted Moloney antiserum was added. To the remaining three or four tubes, normal serum was added instead. All tubes were incubated for 20 min at 37°C. After the incubation 0·05 ml of guinea-pig complement was added to the two tubes incubated with antiserum, and to two of the tubes incubated with normal serum; to the remaining one or two tubes, Eagle's medium was added instead of complement. After the second incubation at 37°C for 30 min, the cells were washed by centrifugation at 3000 rev/min for 1 min, resuspended in 0·1 ml of Eagle by the aid of a Pasteur pipette, admixed to 5×10^7 sheep erythrocytes suspended in 0·8 ml of 0·5% agarose in Eagle at 42°C, and plated in a Falcon plastic Petri dish of 5 cm diameter. Incubation, addition of antigen and enumeration was done in the usual way (Jerne *et al.*, 1963; Celada & Klein, 1967). For each mouse the fraction of PFC surviving the antiserum treatment was calculated with the formula shown in Tables 1 and 2. The data obtained after normal serum and complement (C), and normal serum and Eagle treatment were pooled since they were not significantly different.

In some experiments a suspension of 3×10^7 cells from the portion of the spleen which had not been utilized in the test was transferred to each of a group of syngeneic, 700 r irradiated mice. The recipients were challenged with 10^8 SRBC i.p. and their spleens examined for anti-Moloney serum susceptibility 1 week later.

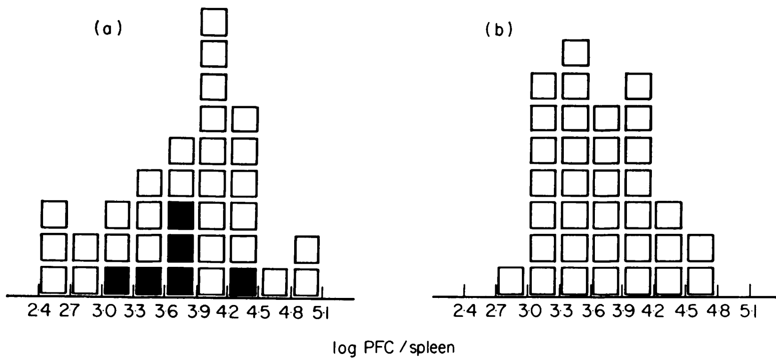

FIG. 1. Distribution of experimental (a) and control mice (b) with respect to their ability to produce anti-sheep erythrocytes PFC. Ordinate: number of mice with a given number of PFC (each square represents one mouse). Abscissa: $\log_{10}$ PFC/spleen. Among the experimental mice, solid squares designate high sensitivity to anti-Moloney serum.

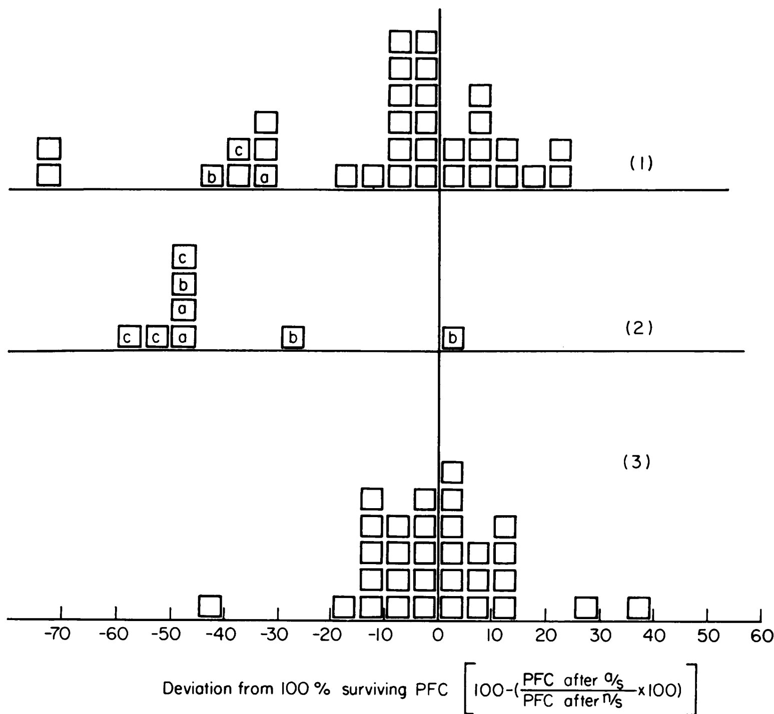

FIG. 2. Frequency of distribution in three populations: (1) MLV-infected mice, (2) secondary recipients of antiserum-sensitive spleen cells from selected donors and (3) control mice. Each square represents one mouse. In (2) mice transferred a/s sensitive cells from three MLV-infected mice (donor, recipient designated with the same letter).

RESULTS

Fig. 1 shows the distribution of the control and experimental mice with respect to their capacity to produce a primary IgM immune response (PFC) against sheep erythrocytes. The data from mice of various strains were pooled in this graph.

TABLE 1. Effect of pre-treatment with antiserum (anti-Moloney) on the number of PFC found in spleen cell suspensions from control mice

Experiment No.	Strain	PFC (average/plate)*			Surviving fraction
		(a) a/s+C	(b) n/s+C	(c) n/s+Eagle	$\frac{a}{(b+c)/2} \times 100$
1	A/Sn	59	60	ND	99
1	A/Sn	47	49	ND	95
1	A/Sn	33	30	ND	112
1	A/Sn	16	15	ND	103
2	A/Sn	27	21	32	106
2	A/Sn	65	51	72	105
2	A/Sn	28	27	42	85
2	A/Sn	26	29	27	90
3	A/BY × DBA/2	49	42	54	103
3	A/Sn	72	90	85	82
3	C3H × C57BL	86	106	90	86
4	C57BL	204	187	180	89
4	C57BL	267	262	261	102
5	Leaden	17	21	16	90
5	Leaden	40	23	20	89
6	A/Sn	17	15	21	139
7	A/Sn	72	87	82	85
7	A/Sn	50	91	88	55
8	A × Leaden	240	248	228	99
9	A × Leaden	50	56	35	102
9	A × Leaden	89	105	100	87
9	A/Sn	143	135	164	99
10	A/Sn	161	85	127	114
10	A/Sn	98	85	85	115
11	A/Sn	83	75	78	106
11	A/Sn	57	43	62	107
12	A/Sn	137	135	134	101
13	A/Sn	176	182	178	98
13	A/Sn	85	88	92	95
14	A/Sn	49	49	51	98
14	A/Sn	27	20	18	130

Experiments done on the same day are designated by the same number (1–14). Each horizontal line corresponds to one mouse. ND, not done.

*Experiments 1–9 had 2 *a* plates, 2 *b* plates, and 1 *c* plate; Experiments 10–12 had 2 *a*, 2 *b* and 2 *c* plates.

The susceptibility of PFC to anti-Moloney serum and complement is illustrated in Tables 1 and 2. Eight of thirty-three experimental mice (24·2%) reacted with a considerable decrease of the PFC to the treatment with Moloney anti-serum (surviving fraction between 28 and 69%) whereas only one of thirty-one control animals behaved in this way.

Fig. 2 shows the frequency distribution of three populations: the controls, the experimental mice, and the irradiated recipients of the antiserum sensitive spleen cell suspensions from the three selected experimental mice. A test of symmetry (Snedecor, Statistical

TABLE 2. Effect of pre-treatment with antiserum (anti-Moloney) on the number of PFC found in spleen cell suspensions from mice injected with MLV at birth

Experiment No.	Strain	PFC (average/plate)*			Surviving fraction
		(a) a/s+C	(b) n/s+C	(c) n/s+Eagle	$\frac{a}{(b+c)/2} \times 100$
1	A/Sn	13	46	ND	28
1	A/Sn	5	4	ND	122
1	A/Sn	14	13	ND	110
1	A/Sn	5	5	ND	90
2	A/Sn	44	44	53	92
2	A/Sn	22	21	18	110
2	A/Sn	29	26	29	95
2	A/Sn	12	10	12	104
3	ABY × DBA/2	32	45	63	61
3	C3H × C57BL	57	71	46	92
3	A/Sn	41	44	28	107
4	Leaden	144	202	215	69
5	Leaden	51	75	81	67
6	A/Sn	400	317	295	121
7	A/Sn	51	54	71	86
7	A/Sn	313	446	509	67
7	A/Sn	265	286	349	91
8	A × Leaden	322	324	326	99
8	A × Leaden	208	185	228	103
9	A × Leaden	345	346	393	96
9	A/Sn	134	173	118	112
10	A/Sn	160	131	127	121
10	A/Sn	328	358	307	99
11	A/Sn	52	58	59	90
11	A/Sn	61	58	53	110
12	A/Sn	149	146	170	95
12	A/Sn	63	102	107	55
12	A/Sn	136	151	156	89
13	A/Sn	58	69	117	80
13	A/Sn	71	67	79	97
13	A/Sn	29	44	48	63
14	A/Sn	12	36	39	33
14	A/Sn	23	20	19	115

Experiments done on the same day are designated by the same number (1–14). Each horizontal line corresponds to one mouse. ND, not done.

*Experiments 1–9 had 2 *a*, 2 *b* and 1 *c* plates; Experiments 10–12 had 2 *a*, 2 *b* and 2 *c* plates.

Methods, 4th edition, p. 175) showed that the distribution of the MLV infected population differed significantly from normality (g_1, the measure of skewness $= -1{\cdot}217$, $t = -2{\cdot}76$ for df $= \infty$, the 1% level of t being $\pm$ 2·57). The asymmetry is due to the six spleens at the

far left with a high sensitivity to the anti-Moloney serum. This group appears as a distinct sub-population among the experimental mice. The result of the transfer experiment is consistent with the test in the corresponding primary host (Fig. 2): seven out of eight recipients of Moloney sensitive spleen cells also showed a decrease in the number of plaque forming cells when their own spleen cell suspensions were exposed to anti-Moloney serum.

DISCUSSION

The results demonstrate that in a proportion of mice injected at birth with Moloney virus, long before there is any sign of leukaemic disease, a high number of IgM antibody forming cells stimulated by heterologous antigen (SRBC) can be inhibited *in vitro* by exposure to Moloney specific antiserum and complement. We have no direct proof that this is due to the actual killing of the antibody forming cells, although this is by far the most likely explanation.

We have no information on the reasons why only one-fifth of the experimental animals exhibited the phenomenon of PFC susceptibility to anti-Moloney serum. Susceptible animals were of different strains, and their total PFC response was situated in the middle of the distribution (see Fig. 1). This fact excludes errors of evaluation due to small sampling population, but does not tell us whether the mice in question were nearest to the outbreak of symptomatic leukaemia, a condition known to interfere with a normal immune response.

Did the antiserum-sensitive antibody forming cells synthesize the Moloney specific antigen under instructions from the viral genome? The persistence of the susceptibility of antibody forming cells after their adoptive transfer points in this direction. If so, then the presence and function of a potentially oncogenic viral genome does not necessarily prevent the cell from performing a complicated series of steps, in line with its normal maturation and differentiation programme. Unfortunately we have no direct proof that the cells actually synthesize the antigen and contain the virus or its genome, although investigations are in progress to elucidate this point.

One alternative would be passive adsorption of the Moloney specific antigen onto the surface of the antibody forming cell. Passive cytotoxicity requires a high concentration of antigen, however, and it is hard to visualize how this could be achieved by adsorption alone, particularly if one considers the persistence of sensitivity after transfer to a new recipient, previously unexposed to the virus.

ACKNOWLEDGMENTS

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NOTE

After the experiments reported here were completed, a paper by Cremer, Dee & Lennette (1969) came to our attention. By the fluorescent antibody technique applied to tissues of rats infected by Moloney virus, the authors detected some lymphoidal cells with staining characteristics indicating both antibody and virus or viral-associated antigen production.

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