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RESEARCH STUDIES OF FACTORS INDUCING RESISTANCE TO TRANSMISSIBLE MOUSE LEUKEMIA

by

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First Quarterly Report

In the period between October 1st, 1952 and January 1st, 1953, six experiments have been performed. These were given serial numbers 1-6, preceded by "CD" (Camp Detrick), as well as numbers in the series of experiments that have been performed in this laboratory, preceded by C.S.H. (Cold Spring Harbor).

In all these experiments the mice were young males from strain C58, with a pedigree of over 95 generations of brother by sister matings. The transplanted leukemia has been line I_b, or its sub-branch I_{ba}; the number following this designation indicates the number of successive C58 hosts in which this population of leukemic cells has been grown since its origin from a case of spontaneous lymphatic leukemia that developed under natural conditions in 1929.

The degree of induced resistance that can be detected is limited by the size of the challenging dose: the smaller the dose that will kill all the controls, the weaker the resistance detectable.

The primary criterion of resistance is indefinite survival after all the controls have died. A second criterion of resistance is the lengthening of the interval before death as compared with the controls. Thus the greatest possible uniformity of all the mice in an experiment has basic importance. In all experiments, equal numbers of treated and control mice have been used and in the groups to be compared critically each litter is equally represented.

CD1 and CD2.

Will 0.3% polyethylene glycol in the 0.9% saline which is routinely used as suspending medium for the leukemic cells from minced spleens, reduce the variability in the interval before death of the inoculated host and eliminate occasional survivors from very dilute (4^{-8} x standard) doses?

These two experiments, covering doses of 4^{-5}, 4^{-6}, 4^{-8} and 4^{-11} x "standard" show a slight hastening of death and a possible slight increase in spleen size in the glycol groups as compared with their saline controls. But with the highest dilution of the dose the occasional survivor is not eliminated. The use of glycol does not offer enough practical advantage to justify its routine use.

CD3.

Will a resistant state be induced by treatment with whole dog's blood 1-3 days before challenge with line I_b leukemia? This question was raised by the various parallels between the resistance successfully induced by certain procedures against line I_b leukemia and Dr. Victor's studies on inducing resistance. A single treatment with fresh tissue from a mouse strain unrelated to the strain of susceptible hosts enables the mouse to survive a dose of leukemic cells 100% lethal to the untreated controls. Would tissue (blood) from a different order of mammals (instead of a different strain of the same species) induce a similar phenomenon? The results give no evidence of any delay in death resulting from the treatment with dog blood, whether this was given one, two, or three days before the challenge dose. Indeed one mouse in each of the treated groups died before the first control.

CD4.

To eliminate possible effects of freezing upon the dog blood, an experiment was performed with dog blood drawn immediately before treating the mice. In this case the challenging dose of leukemic cells was reduced to 4^{-8} x standard, and given one and two days after the treatment.

These modifications (the elimination of freezing and a reduction of the challenging dose to 1/16) did not change the result. Although two delayed deaths appeared in group A (treatment 48 hours before challenge) two negatives occurred in the controls from the same litters and another control death was equally delayed.

CD5.

The leukemic cells of line I_b themselves produce a substance that directly and rapidly induces resistance in the host. This has been demonstrated by treatment with leukemic cells after moderate heating, or disruption by sonic vibration.

This experiment attempted to determine whether protective action could be found in the suspending medium after the leukemic cells had been gently agitated. The results were inconclusive, in that after centrifugation at 2000 x G for 30 minutes enough leukemic cells remained in the supernatant to significantly shorten the interval before death as compared with the controls, when challenged in two days with a highly dilute dose. In spite of this, three of 25 mice treated with the supernatant survived and none of the 25 controls. Considering this experiment by itself these survivors might seem significant, but in other experiments controls have survived this same dilute dose (4^{-8}), as in Exp. CD4.

CD6.

The resistance induced by heated line I_b cells may resist a highly dilute dose of unheated leukemic cells given at once; after 48 hours the resistance is considerably stronger, but then soon weakens. After 8 days survival is very doubtful. This stands in contrast to the persistence of resistance developed after a very few untreated leukemic cells have been resisted and raises the question whether the mechanism in the two cases is different or whether the difference depends on the amount of protective material available in the heated cells, compared with unheated cells. Although vastly more heated cells are inoculated, they do not multiply, whereas the unheated cells do multiply and establish histologically demonstrable lesions before succumbing to the resistance mechanism. This experiment attempted to increase the amount of protective material by repeated doses of heated cells. Groups of litter-mate hosts were given 1, 2, and 3 daily treatments with line I_b cells heated for 14 minutes in a 46° C water bath and challenged 9 days after the last treatment with a dose (4^{-6}) that previously had been resisted at 8 days after treatment with heated cells by only 3 out of 20 mice.

In the present experiment only one mouse died of the 10 receiving three treatments of heated cells but the single treatment groups gave unexpectedly high survival--7 out of 10. Five controls on the first of the three treatments were challenged (4^{-6}) in 2 days and all survived, while all the 5 controls on the challenging dose died from this particular suspension of unheated cells.

Evidently 9 days was too soon to test the differences between the persistence of 1 vs. 3 treatments with heated cells.

Protocols for each experiment follow.

Comparison of 0.3% polyethylene glycol (abbreviated = "glyc") in 0.9% saline with 0.9% saline as suspending medium for high dilutions of line I_b leukemia.

Donor spleen (#1682, CSH 2113, I_b 2067) was cut into two parts, each part weighed and minced separately and suspended with the respective solutions; 2.3 cc per gram of spleen = "standard suspension." Each standard suspension at once reduced to 4^{-3}, and for each subsequent dilution the saline control was diluted immediately after the "glyc" suspension. All suspensions originally refrigerated and maintained cold in crushed ice.

Dilution schedule

Standard - 1 g. spleen to 2.3 cc. suspending medium

4^{-3} = .05 cc std + 3.15 cc "glyc" or saline

4^{-5} = .20 cc 4^{-3} + 3.00 cc " " "inoculate 5 mice; each medium

4^{-8} = .10 cc 4^{-5} + 6.30 cc " " " " 10 " " "

4^{-11} = .10 cc 4^{-8} + 6.30 cc " " " " 10 " " "

Mice Strain C58, males, born 8/17-8/23, 1952; equal number from each litter in "glyc" and saline groups.

Saline controls inoculated immediately after "glyc" group with same dilution.

All inoculations between 12M and 12:50 P.M.

Results: Intervals before death by 1/4 days

	7th	8th	9th	10th	11th	12th	13th	15th
Dilution 4^{-5}								
"Glyc"	1 3	1						
Saline	2 1 1	1						
Dilution 4^{-8}								
"Glyc"			1 3	6				
Saline				2 1 1 1	5			
Dilution 4^{-11}								
"Glyc"					2	6 1	1 neg.	
Saline						1 5	2	1
							1 neg.	

Spleen size (cm.) averages

	Dilution	Length	Width
4^{-5}	"glyc"	2.88	.84
	saline	2.96	.80
4^{-8}	"glyc"	3.06	.93
	saline	2.90	.91
4^{-11}	"glyc"	2.92	.93
	saline	2.71	.85

Exp. CD2 CSH 2124 I_{ba} 2069. 10/16/52

Further test of 0.3% polyethylene glycol as suspending medium using dilution 4^{-6} this being the dilution to be used chiefly in subsequent experiments.

Procedure as in CD1.

Two parts of same leukemic spleen (Donor #41819 from CSH 2119 I_{ba} 2068) separately weighed, minced and suspended in respective solutions. All vials kept in crushed ice.

Dilution schedule.

Standard = (1 g. spleen to 2.3 cc. suspending medium)

4^{-3} = .05 cc Standard + 3.15 cc. medium

4^{-6} = .05 cc 4^{-3} + 3.15 cc. suspending mediun inoculated .2 cc. per mouse; 10 mice each medium.

Results:

Interval before death by 1/4 days

Dilution 4^{-6}

	8th			9th			
	.	.	.	.	.	.	
"glyc"	4	1	5				
saline	2	2	5	1			

Spleen size in cm. averages

	Length	Width
"glyc"	3.03	.94
saline	3.01	.92

Experiment CD3 CSH 2130 I_{ba} 2070 10/23/52

Treatment with frozen, fresh, heparinized dog blood (whole) .4 cc per mouse, 1 or 2 or 3 days before challenging with line I_b leukemia--dose 4^{-6} x standard.

Each litter (strain C58 ♂s born 8/31-9/13/1952) divided equally between four groups = i.e. challenged after 1 day, 2 days, 3 days and untreated controls.

Dilution schedule.

Spleen from ♂42062 in CSH 2124. I_{ba} 2069, dose 4^{-5} x Standard on 10/16/52

Standard = 2.3 cc. saline to 1 g. spleen weight.

4^{-3} = .05 cc Std. + 3.15 cc. saline

4^{-6} = .15 cc 4^{-3} + 9.45 cc. saline inoculated 40 mice: 0.2 cc per mouse

Order of inoculation (5 mice per box)

1st box of each of the four groups, then 2nd box of each group. All inoculations within 20 minutes (4:30-4:50 P.M.).

Results:

Time of death by 1/4 days

Days between treatment and challenge	7th	8th	9th	
3	1	3 4	2	
2	1	3 3 1	1	(1* negative)
1	1	3 3 1	1	(1 killed by accident)
Untreated controls		4 4 1	1	

* Reinoculated 11/6/52 (4^{-6}) died in 8 d. showing record of 1st inoculation probably in error--possibly into bladder. Survival of one lethal dose ensures survival of a second one.

Spleen size (cm.) averages

	Length	Width	Range of lengths
3 days	2.98	.96	2.8 - 3.1
2 "	2.92	.90	2.8 - 3.2
1 day	2.88	.99	2.8 - 3.2
Controls	2.90	.94	2.7 - 3.2

Experiment CD4 CSH 2142 I_b 2073 11/13/52

Treatment with heparinized dog blood, immediately after withdrawal from dog. .4 cc. per mouse, 24 hours (11/12/52) and 48 hours (11/11/52) before challenging with I_b 4^{-8} x Standard. Each litter of C58 's equally represented in each of these two treated groups and the untreated controls, i.e., 4 sets of 3 boxes each. Each group included 4 boxes of five mice.

Inoculation order: 1st box of each group; 2nd box of each group, etc.

A = treated 48 hrs. before challenge; B = treated 24 hrs. before challenge; C = controls.

Dilution schedule.

Spleen from 42704 from CSH 2139 I_b 2072 11/6/52--dose 4^{-6} x standard. All vials kept in crushed ice; saline pre-cooled.

Standard - by measurement table.

4^{-2} = .10 cc Std. + 1.5 cc saline

4^{-5} = .05 cc 4^{-2} + 3.15 cc saline

4^{-8} = .15 cc 4^{-5} + 9.45 cc saline - inoculate first 2 boxes of each group

4^{-8} = .15 cc 4^{-5} + 9.45 cc saline - inoculate second 2 boxes of each group

All mice inoculated between 11:12-11:52 A.M.

CD4 - Distribution of deaths after Ib 4^{-8} on 11/13/52

Box	9th	10th	11th	12th
1A	1 3	1		
1B	3 1 1			
1C control	1 3			1
2A	2 1 1	1		
2B	2 1 1	1		
2C control	1 2 2			
3A	2 1 2			
3B	1 3	1		
3C control	1 1 2		1	
4A	1 2			1 1
4B	2 1	1 1		
4C control	1 1	1		2 survivors
Totals				
A	6 7 3	1 1		1 1
B	1 8 4 3	2 2		
C controls	4 7 4	1	1	1 (2 survivors)

Distribution of Spleen Length

	2.- .6	.7	.8	.9	.0	3.- .1	.2	.3	.4	.5	.6 cm	Spleen Length Averages	Width (cm)
A		1	*1		1	4	3	6	4			3.19	.97
B					1	4	5	5	2	2	1	3.26	.98
C controls	1	1		2	2	3	5	2	1	1		3.09	.95

* Delayed deaths

CD5 CSH 2149 I_{ba} 2074 11/20/52, and CSH 2152 I_b 2075, 11/27/52

Treatment with supernatant from line I_b diluted to 4^{-1} x standard after agitation for 30 minutes on mechanical shaker and centrifugation 2000 x G for 30 minutes; challenged in two days with a dose 4^{-8} x standard. 5 boxes of 5 treated mice compared with 5 boxes of litter-mate controls.

		Time of death by 1/4 days				Average spleen	
		7th	8th	9th	10th	Length	Width
11/20	Supernatant	1 2	1	1		2.98	.88
	Controls			1 1 1 2		2.84	.94
11/27	Supernatant	1 2	1 1			2.82	.84
	Controls			1 1 2 1		2.86	.80
11/27	Supernatant		1 1 2	(1 survived)		2.92	.85
	Controls			2 2 1		2.98	.90
11/27	Supernatant	1	3		(1 surv.)	2.95	.85
	Controls			1 2 1	1	2.98	.90
11/27	Supernatant	2	1 1		(1 surv.)	2.72	.90
	Controls			1 1 1	2	3.00	.90
Totals		1 2 2 4	6 1 3 2	1	(3 surv.)	2.87	.86
				3 7 6 6	1 2	2.93	.88

CD6 CSH 2163 I_b 2078: Heat series 128.

Line I_b 2076 - Spleens from CSH 2156, heated 46° C. 14 minutes .25 cc. of standard suspension in saline, given on 1 or 2 or 3 successive days.

Challenged (4^{-6}) on 9th day after the last heated cells.

Each litter (males only) equally represented in each of the three treatment-groups and the group of untreated controls.

Results given by litters.

Mothers	Intervals before death--days: 3 treatments	2 treatments	1 treatment	Controls on challenging dose
	A_1	B_1	C_1	D_1
40415	Surv.	Surv.	Surv.	8-
41402	Surv.	10	8+	8 1/2
41571	Surv.	Surv.	Surv.	8+
41868	Surv.	Surv.	Surv.	8+
41129	Surv.	Surv.	8-	8
	A_2	B_2	C_2	D_2
40490	Surv.	13	Surv.	8-
39668	(Surv. (10	8- 8+	8 Surv.	(8- (8-
41209	Surv.	10-	Surv.	8-
41185	Surv.	Surv.	Surv.	8-
Total deaths	1/10	5/10	3/10	10/10
Survival	90%	50%	70%	0%