Demonstration of Mixed Lymphocyte Interaction in Hamsters[1] (37042)

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The Syrian hamster is being used as an animal of choice for testing oncogenic potential of viruses, especially DNA-containing viruses. The cells transformed *in vitro* by DNA viruses become readily transplantable in syngeneic adult hamsters (1). With increasing interest and need to understand tumor–host relationships in hamsters, it is important to study hamster lymphocyte functions. We have previously reported (2) that hamster lymphoid cells can be stimulated *in vitro* with mitogens concanavalin A (Con A) and phytohemagglutinin (PHA)-P to undergo DNA synthesis. The present investigation was undertaken to determine if hamster lymphocytes would react in mixed lymphocyte culture (MLC) reaction. MLC has been demonstrated in the human system (3, 4) and has also been used successfully to delineate strain differences in mouse (5, 6), rat (7, 8), and rabbit (9) systems. The results of this study indicate that MLC reaction can be successfully used to demonstrate histocompatibility differences between two inbred strains of hamsters (CB/Ss LAK and MHA/Ss LAK), which have previously been shown to differ by one or two histocompatibility loci by skin allograft rejection (10, 11).

Materials and Methods. Lymphoid cells. Two inbred strains of Syrian hamsters, MHA/Ss LAK and CB/Ss LAK (Lakeview Hamster Colony, Newfield, NJ), which are histoincompatible with each other, were used as lymphoid cell donors. Lymph node and spleen cells from 2–3 mo old hamsters were prepared separately as previously described (2) and were cultured for 5 days in single or mixed cultures in RPMI-1640 medium (Grand Island Biological Co) supplemented with 10% heat-inactivated fetal calf serum at 37° in an atmosphere of 5% CO_2 and 90% humidity. For one-way stimulation, the cells to be used as stimulating cells were treated with mitomycin C (Sigma Chemical Company, St. Louis, MO) at a concentration of 25 μg/ml for 30 min at 37° as described (12).

DNA synthesis. The cultures were pulsed with 1 μCi of thymidine-methyl-^{3}H (New England Nuclear, sp act 20.2 Ci/mmole) for the final 12 hr of incubation and trichloroacetic acid-precipitable radioactivity was determined as previously described (2). Stimulation ratio was calculated by the following formula:

$$\text{Stimulation ratio} = \frac{\text{cpm } (x \text{ MHA} + x \text{ CB}) \times 2}{\text{cpm } (2x \text{ MHA}) + \text{cpm } (2x \text{ CB})}$$

where x represents the number of cells per culture.

Results. It was first determined if hamster lymphocytes will respond to a known mitogen Con A. The results in Table I, Expt. I show that lymph node cells derived from CB and MHA hamsters could be stimulated by Con A (2 μg/culture). The ratios of stimulation obtained were 82 and 19 for MHA and CB hamsters, respectively, thus confirming our previous observation (2) that hamster lymphocytes can be successfully stimulated to undergo DNA synthesis after Con A stimulation.

Two-way MLC was performed by measuring

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TABLE I. Two-way Mixed Lymphocyte Culture Reaction Using MHA and CB Hamster Lymphoid Cells.

Source of lymphoid cells	Expt no.	No. of lymphoid cells		Mitogen	^{3}H-Thymidine incorporation[a] (counts/min)	Ratio of stimulation
		MHA	CB			
Lymph node	I	1.0×10^6	—	—	2456	
		—	1.0×10^6	—	9114	
		1.0×10^6	—	Con A[b]	201,277	82.0
		—	1.0×10^6	Con A	169,793	19.0
		0.5×10^6	0.5×10^6	—	28,410	4.8
	II	1.0×10^6	—	—	4926	
		—	1.0×10^6	—	1320	
		0.5×10^6	0.5×10^6	—	28,919	9.3
	III	1.0×10^6	—	—	3941	
		—	1.0×10^6	—	2670	
		0.5×10^6	0.5×10^6	—	17,726	5.4
	IV	2.0×10^6	—	—	2943	
		—	2.0×10^6	—	5558	
		1.0×10^6	1.0×10^6	—	24,186	5.7
	V	2.0×10^6	—	—	2968	
		—	2.0×10^6	—	1882	
		1.0×10^6	1.0×10^6	—	12,632	5.2
Spleen	I	1.0×10^6	—	—	25,003	
		—	1.0×10^6	—	29,663	
		0.5×10^6	0.5×10^6	—	93,749	3.4
	II	1.0×10^6	—	—	26,117	
		—	1.0×10^6	—	34,279	
		0.5×10^6	0.5×10^6	—	106,705	3.5

[a] Average of 2 or 3 cultures.

[b] Con A = lymph node cell cultures stimulated with 2 μg Con A for 72 hr.

DNA synthesis of lymphoid cells derived from CB and MHA hamsters at the end of the 5 day culture period. The results of 7 experiments, presented in Table I, show that histocompatibility differences between these 2 strains of hamsters can be detected by the MLC test. The stimulation ratios obtained in two-way MLC tests ranged from 4.8 to 9.3 in 5 experiments with lymph node cells. The results in Table I also show that MLC can also be performed using spleen cells from CB and MHA hamsters. The stimulation ratios with spleen cells were 3.4 and 3.5. These results clearly indicate that MLC can be successfully applied to detect strain differences in hamsters.

The data on one-way stimulation in MLC using MHA and CB lymph node cells are presented in Table II. MHA lymph node cells treated with mitomycin C were found to stimulate CB lymph node cells, as shown by the stimulation ratios of 3.6 and 3.7. Similarly, lymphocytes from CB hamsters after treatment with mitomycin C successfully stimulated MHA lymph node cells. The ratio of stimulation in this case was 4.5. These results show that both CB and MHA lymphocytes are functional in the one-way MLC test.

Discussion. The results presented in this study clearly show that the MLC reaction can be successfully employed to demonstrate strain differences in hamsters. When lymph node or spleen cell cultures of MHA and CB inbred strains of hamsters are cocultivated for a period of 5 days, the cells undergo blastogenic transformation, incorporating significantly increased amounts of ^{3}H-thymidine. This is evident from the two-way MLC–stimulation ratios of 6.1 (av of 5 tests) for lymph node cells and 3.4 (av of two tests) for spleen cells. Significant stimulation ratios were also obtained in the one-way MLC reaction. In transplantation studies (10, 11, 13), it has been shown that the median sur-

TABLE II. One-way Mixed Lymphocyte Culture Reaction Using Hamster Lymphocytes.

No. of lymph node cells		^{3}H-Thymidine incorporation[a] (counts/min)	Ratio of stimulation
MHA	CB		
2.0×10^6	—	2943	
—	1.0×10^6	659	
2.0×10^6 (M)[b]	—	595	
4.0×10^6 (M)	—	1498	
—	2.0×10^6 (M)	1014	
2.0×10^6 (M)	1.0×10^6	4455	3.6
4.0×10^6 (M)	1.0×10^6	7898	3.7
2.0×10^6	2.0×10^6 (M)	17,610	4.5

[a] Average of 2 or 3 cultures.

[b] (M) = mitomycin C treated.

vival time of skin homografts exchanged between MHA and CB isogenic strains of hamsters rarely exceeds 11–12 days whereas intrastrain grafts are seldom rejected. This evidence suggests that these two strains of hamsters differ by strong histocompatibility antigens. Previously, all attempts by Fernald and Metzgar (14) to demonstrate MLC using lymphoid cells from MHA and golden Syrian hamsters were unsuccessful, probably because these hamsters differ by only minor histocompatibility antigens. Our *in vitro* MLC results correlate well with the above *in vivo* observations of Billingham and associates (10, 11, 13). The successful use of MLC in hamsters will be of great help in characterizing and developing new stocks of inbred hamsters for use in studies on tumor transplantation and viral oncogenesis.

Summary. Strain differences between two inbred strains of hamsters (CB/Ss LAK and MHA/Ss LAK) have been successfully demonstrated using mixed lymphocyte culture (MLC) reaction. The average stimulation ratio in a two-way MLC was found to be 6.1 when lymph node cells were used. The lymphocytes from spleen cells were also reactive in MLC. In the one-way stimulation test, lymphocytes from CB hamsters stimulated lymphocytes from MHA hamsters and vice versa.

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