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Immunogenetic Studies of H-2 Antigens of MCG 3 Tumour Cells

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MCG 3 is a B 10(H-2^b) sarcoma induced as a solid tumour with 0.2 mg of methylcholantrene in our laboratory. The ascites form obtained was used to study the expression of H-2 antigeneic specificities in a postlabelling radioassay.

H-2D.2, H-2K.33 (private specificities of H-2^b) and H-2.28, H-2.35 (public specificities) were normally expressed for MCG 3 while H-2.5, H-2.29, H-2.36 (public specificities) were abnormally absent.

These results were confirmed by quantitative absorption analysis using MCG 3 and positive-negative normal lymphoid cells for a particular specificity.

There is increasing evidence pointing to the possibility of tumour specific transplantation antigens (TSTA) being foreign histocompatibility antigens (3, 5, 7). The mechanism of this new antigenicity is still controversial (2, 6).

In this report the pattern of reactivity of twenty four H-2 alloantisera with tumour cells of a chemically induced B 10(H-2^b) sarcoma (MCG 3) using a postlabelling radioassay is shown.

Materials and Methods

MCG 3 is a B 10(H-2^b) sarcoma induced as a solid tumour with 0.2 mg of methylcholantrene in our laboratory. The primary tumour appeared 5 months later and was serially transplanted in syngeneic mice to obtain the ascite form.

Mice. Adult mice of the following strains were used: B 10(H-2^b), B 10D$_2$ (H-2^d), B 10A(H-2^a), C 57B1/6(H-2^b) and CBA(H-2^k). They were used for passage of the tumour cells (B 10) and to perform absorptions of alloantisera with positive or negative lymphoid cells for a particular specificity.

Antisera. A panel of H-2 alloantisera with restricted and defined specificity (kindly provided by Dr. Ray, of The National Institute of Health, Bethesda, Maryland) and poliespecific antisera anti H-2^k,

anti H-2^b, anti H-2^a and anti H-2^{t3} were used.

Tumour cells. The following mouse tumour cells line of different aetiology and origin were used: the TLC 5 lymphoma from CBA/H (H-2^k); the P815 X_2 sarcoma from DBA/2(H-2^d); the GIL IV lymphoma from C57B1/6(H-2^b) and the MCG 3 sarcoma from B 10(H-2^b).

Absorption procedure. To characterize expected and unexpected reactions, H-2 alloantisera were absorbed with positive and negative lymphoid cells for a particular specificity. Spleen and lymph node cells were used as absorbing cells from 2×10^7 to 80×10^7, keeping the amount of antiserum constant (50 μl of 1/10 dilution).

Microradioassay. The expression of H-2 antigenic specificities was studied in a post-labelling assay. Details of the procedure have been published elsewhere by GARRIDO *et al.* (6). In brief, 4×10^4 tumour cells suspended in MEM supplemented with 10 % FCS are incubated with 10 μl of specific antiserum in the presence of complement for 30 min at 37° C in a humidified 5 % CO_2 air atmosphere, followed by measuring ^{14}C-thymidine uptake in a postlabelling assay.

Results and Discussion

Table I shows the pattern of cytotoxicity in MCG 3 with a panel of H-2 alloantisera of restricted specificity and poliespecific anti H-2.

The results are expressed as the percentage of reduction of thymidine uptake in test samples compared with control samples with complement and no antibody. H-2D.2, H-2K.33 (private specificities of H-2^b) and H-2.28, H-2.35 (public specificities) were normally expressed while H-2.5, H-2.29 and H-2.36 were abnormally absent.

MCG 3 seem to be Thy 1.1, Thy 1.2, Ly 4.2 and Ia negative tumour (table I). Other cytotoxicity reactions were also obtained with various poliespecific anti H-2 sera because, according to the NIH catalogue, they contain antibodies other than the specificity and kill the H-2^b cells.

These results were confirmed by quantitative absorptions with MCG 3 and positive-negative lymphoid cells for a particular specificity except. H-2.28.

Anti H-2.33 and H-2D.2 sera were absorbed as expected with increasing numbers of C57B1/6(H-2^b) normal lymphoid cells and MCG 3 tumour cells (cytotoxicity negative against GIL IV, a positive tumour for H-2.33 and H-2D.2) but they were not absorbed with B 10A (H-2^a) (cy-

Table I. *Cytotoxicity obtained with mouse alloantisera defining H-2 K H-2D, Ia and Thy. 1 antigens in MCG 3 sarcoma.*

H-2^b specificities		Non H-2^b specificities		Others	
Sera	Cytotoxicity (%)	Sera	Cytotoxicity (%)	Sera	Cytotoxicity (%)
Anti H-2D.2	53	Anti H-2.1	0	Anti Ly 4,2	0
Anti H-2.5	0	Anti H-2.3	0	A.TH anti A.T1	0
Anti H-2.28	51	Anti H-2D.4	0	Anti Thy 1.1	0
Anti H-2.29	0	Anti H-2.8	0	Anti Thy 1.2	0
Anti H-2.35	60	Anti H2.11	6	BALB/c anti CBA/H	0
Anti H-2.36	0	Anti H-2.13	0	B10 D_2 anti B1OA	0
Anti H-2K.33	56	Anti H-2K.19	0	B1OBR anti HTT	54
		Anti H-2.25	0	CBA/H anti C57BLB6	53
		Anti H-2K.31	0	C57B1/ anti B1OBR	0

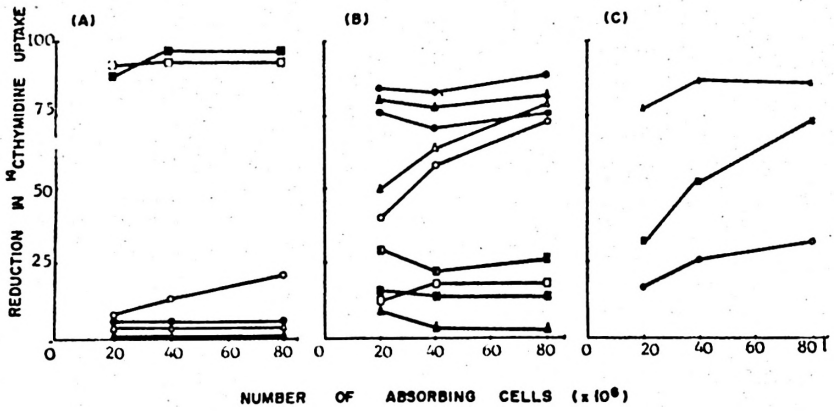

Fig. 1. *Cytotoxicity of H-2 antigens.*
A) Cytotoxicity of anti H-2^k.33 and H-2D.2 against GIL-IV (a positive tumour for these specificities). The antiserum D-33 was absorbed by cells of C57BL/6 (○) and MCG 3 (△) but not by B 10A (□) (negative control). The antiserum anti H-2D.2 was absorbed by MCG 3 (▲), C57BL/6 (●) (positive control) and not by B10 A (■) (negative control). *B*) Cytotoxicity of anti H-2.36, H-2.35 and H-2.29 against P815X$_2$ tumour cells. The antiserum D-36 was absorbed by normal cells of B10A (□) (positive control) and not by CBA/H (○) and MCG3 (△). The cytotoxicity of anti H-2.35 was absorbed as expected by B 10A cells (■) (H-2.35 positive) and MCG3 (▲) but not by CBA/H(●) (H-2.35 negative). The anti H-2.29 was absorbed with B 10A (□) (positive control), MCG3 (△) and CBA/H (○) (negative control). *C*) The ortotoxicity of anti H-2.5 in TLC 5 lymphoma (H-2^k), a positive tumour for H-2.5, was absorbed by CBA/H(H-2^k) (○) and not by MCG3 (H-2^b) sarcoma (△) and B 10D$_2$ (H-2$_d$) (□) normal lymphoid cells.

totoxicity positive) (fig. 1 *A*). Anti H-2.35 was absorbed with normal lymphoid cells of B 10A and MCG 3 tumour cells (cytotoxicity negative against P815X$_2$, a positive tumour for H-2.35) but it was not with CBA/H(H-2^k) (cytotoxicity positive) (fig. 1 *B*). The same pattern of reaction was obtained with H-2.5, H-2.29 and H-2.36 (fig. 1 *B, C*).

The presence of foreign H-2 like specificities on the surface on tumour cells as well as on virus-infected cells has recently been pointed out (3, 5). The absence of four H-2 antigenic specificities in a H-2^d sarcoma (MCG 4) has been recently established (4). It seems unlikely that a direct modification induced by methylcholanthrene, endogenous virus, or additional protein (1) may be responsible for the absence of different defined cell surfaced antigens.

Resumen

Se muestran los resultados del tipaje de un nuevo tumor MCG 3 inducido en la cepa de ratones B10 (H-2^b) con 0,2 mg de 3-metilcolantreno, usando un radioensayo de marcaje final, empleando aloantisueros anti H-2 y complemento.

Los antígenos obtenidos muestran que el tumor MCG 3 expresa las especificidades privadas (H-2D.2 y H-2^k.33) características de la cepa B10. Asimismo, analizando las especificidades públicas que debía expresar de acuerdo con el haplotipo de origen, se encuentra la presencia de H-2.28 y H-2.35 y la ausencia de H-2.5, 2-2.29 y H-2.36.

Para confirmar la presencia o ausencia de estas especificidades H-2 se realizan absorciones específicas de los antisueros con células del tumor MCG 3 y células linfoides normales de distintas cepas de ratones.

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