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Relationship Between Foreign H-2-Like Antigens on MCG4 Sarcoma and the *in vivo* Rejection of this Tumor by Syngeneic and Semisyngeneic Mice

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MCG4, a BALB/c sarcoma (H-2^d), that abnormally expresses H-2^k and H-2^b-like antigens, regressed spontaneously when inoculated in BALB/c and in BALB/c hybrids F_1. A relationship was found between the anti-H-2^k and/or anti-H-2^b activity detected in the sera and spleen cells of the regressor mice and their capacity to reject the tumor.

Several papers have recently reported that some murine tumors express foreign H-2 like antigens (1, 2, 6, 7). In certain tumors cross-reactions between those foreing antigens and TATA (Tumor Associated Transplantation Antigens) have been demonstrated (3), suggesting that those molecules appearing on the tumor cell membrane could mediate against neoplasias by a mechanism of immune surveillance (4). In this paper we report data that favour this hypothesis.

Abbreviations: CDC, complement dependent cytotoxicity; CMC, cell mediated cytotoxicity.

Materials and Methods

The following strains of mice were used: BALB/c and B10 D2 (both H-2^d); C57 Bl/6 and B10 (both H-2^b); CBA/H and B10 BR (both H-2^k). Different hybrids of BALB/c were also used; BALB/c × B10 D2 F_1 (C × D2F_1); BALB/c × B10 BR F_1 (C × BRF_1) and BALB/c × CBA/H F_1 (C × HF_1). MCG4, a chemically induced sarcoma of BALB/c (H-2^d) origin was maintained in ascitic form by intraperitoneal passages in syngeneic receptors. Groups of 10 mice were formed with BALB/c and with each type of hybrid. 2.5×10^6 MCG4 cells were given subcutaneously to each mouse. Six weeks

later some of the mice had died and the rest were tumor free. Regressors were bled and sera tested for complement dependent cytotoxicity (CDC) against MCG4 and normal lymphoid cells. The reactivity of sera was measured by a ^{51}Cr release assay described elsewhere (8). Spleen cells of regressors, restimulated by coculturing for 6-7 days with Mitomycin C treated MCG4 cells, were tested for cell mediated cytotoxicity (CMC) with MCG4 and ConA-activated lymphoblast labelled with ^{51}Cr (5).

Results

Table I summarizes the following results: MCG4 regressed spontaneously in 5 BALB/c and in 6 C × D2F$_1$, showing in both groups humoral and cellular activity against MCG4, H-2^k cells (CBA/H and B10 BR) and H-2^b cells (C57B1/6 and B10). The 10 C × B10F$_1$ mice rejected the tumor. The serum and the immune cells of these mice lysed MCG4 and H-2^k cells but, as expected, they did not lyse H-2^b cells. In C × HF$_1$, MCG4 regressed in 7 mice. The serum and spleen cells of these mice reacted with MCG4 and H-2^b but not with H-2^k cells. The tumor was only rejected by two of ten C × BRF$_1$, showing neither humoral nor cellular anti H-2^k and anti H-2^b activity. However, the spleen cells of these regressors killed MCG4. No cytotoxicity was detected in the different groups of regressors against H-2^d control cells (BALB/c and B10 D2).

Discussion

We have observed that groups of mice with a high level of MCG4 rejection (BALB/c, C × B10F$_1$, C × HF$_1$) showed either anti H-2^b or anti H-2^k humoral and cellular activity, whereas C × BRF$_1$, a group with a low capacity to reject MCG4 exerted neither anti H-2^b nor anti H-2^k activity. This could suggest that the ability to reject MCG4 is related to an immune response against foreign H-2 like antigens expressed on the membrane of the tumor.

However, we must point out that at least two of ten C × BRF$_1$ were capable of rejecting the MCG4 though these regressors did not show any reactivity against H-2^k and H-2^b antigens. Moreover, the spleen cells of these mice killed MCG4 *in vitro*. All this indicates that antigens other than H-2^b and H-2^k were also recognized.

According to all these conclusions, a complex of different antigens seems to be involved in the rejection of MCG4, each one capable of inducing an independent immune response against the tumor. The

Table I. *Specific humoral and cellular activity against MCG4, H-2^b and H-2^k cells exerted by mice that have rejected MCG4 sarcoma.*

Mice	Proportion of regressor mice after inoculation with 2.5 × 10^5 MCG4 cells Regressor/Total of mice	CDC of sera (dilution 1/25) of regressors with MCG4 and normal lymphoid cells				CMC of spleen cells of regressor with MCG4 and ConA-activated normal lymphoblasts (Spleen cells/Target cells = 1/25)			
		MCG4	C57B1/6 (H-2^b)	CBA/H (H-2^k)	BALB/c (H-2^d)	MCG4	B10 (H-2^b)	B10BR (H-2^k)	B10D2 (H-2^d)
		Percentage of ^{51}Cr specific release							
BALB/c	5/10	73	49	51	3	63	48	80	2
C × D2F$_1$	6/10	84	46	53	0	62	42	85	0
C × B10F$_1$	10/10	65	0	48	2	61	3	56	4
C × BRF$_1$	2/10	7	2	3	1	40	4	0	3
C × F$_1$	7/10	62	56	0	4	61	47	0	1

success or failure of the tumor rejection could depend on these diverse immune responses.

Resumen

El MCG4, un sarcoma de BALB/c (H-2^d), que expresa anormalmente antígenos H-2^k y H-2^b similar, regresa espontáneamente cuando se inocula en BALB/c y en híbridos F_1 de BALB/c. Se encuentra relación entre la actividad anti H-2^k y/o anti H-2^b detectada en el suero y las células inmunes de los ratones regresores y la capacidad de éstos para rechazar el tumor.

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