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Inhibition of the Induction of Contrasuppression by Antisera Against Tumor-Associated Surface Antigens on Methylcholanthrene-induced Sarcomas*

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A. Introduction

Contrasuppression is an immunoregulatory T-cell activity that protects *Lyt* 1^+, 2^- T-helper cell activity from suppression. This activity involves both an "induction" (afferent) phase, which requires the activation of an *Lyt* 1^+, 2^- effector T cell by cells in the contrasuppressor circuit [6], and an "effector" (efferent) phase, in which the effector cells or cell-free products secreted by these cells render T_H cells resistant to suppression [4]. Recently we discovered an activity in antisera raised against methylcholanthrene-induced sarcomas from Balb/c mice, which blocks T-cell regulatory activity [3]. These antisera block the afferent as well as the efferent phase of suppression to SRBC in vitro, but only in animals which express the same Igh gene polymorphism as Balb/c (Igh^a). We therefore tested whether these antisera could block the afferent and efferent phases of contrasuppression, and whether this activity had any effect on the growth of tumors in those mice.

B. Materials and Methods

The chemically induced sarcomas, and the antisera against them, were prepared according to procedures described by DeLeo et al. [1, 2]. Suppressor T cells were prepared according to the method of Janeway [5]. Contrasuppressor T cells were prepared according to the method of Green [4]. Contrasuppressor factor ($T_{CS}F$) is a cell-free supernatant collected from in vitro generated T_{CS} cells. Generation of primary anti-SRBC cultures and blocking assays with antisera has been described [3]. Assays for metastasis were performed by injecting 10^5 or 5×10^4 Meth A cells into the right footpad of test animals. After 3–4 weeks, lymph nodes were removed and weighed and examined histologically for evidence of tumor cell growth. Animals positive for metastasis were those which showed tumor cell growth in the popliteal lymph nodes of the left leg, as well as both axilary lymph nodes.

C. Results

Antisera effective in blocking the afferent but not the efferent phase of suppression were tested for their abilitiy to block the afferent and efferent phases of contrasuppression (Table 1). Antisera raised in syngeneic Balb/c mice against Meth A (or other MC-induced tumors, data not shown) were ineffective in blocking the activity of either the $T_{CS}F$, which represents the efferent phase of contrasuppression, or the T_{CS} cells, which represents the afferent phase of contrasuppression. However, antisera raised in semisyngeneic $CB6F_1$ or *Igh* congenic C.B20 mice effectively blocked the activity of the Balb/c T_{CS} cells but not the $T_{CS}F$. Likewise, these antisera were very effective in blocking afferent T_{CS} activity in $CB6F_1$ mice, while they were ineffective in blocking T_{CS} activity in *Igh* disparate mice, reiterating the earlier finding on the nature of

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Table 1. Antisera to Meth A raised in Igh^{b+} mice block contrasuppression

Assay cells[a]	Antisera	Anti-SRBC PFC/culture			
		–[b]	T_s	$T_s+T_{cs}F$	T_s+T_{cs}
Balb/c (Igh^a)	–	1600	300	1400	1200
Balb/c (Igh^a)	Balb/c anti-Meth A	1400	400	1400	1400
Balb/c (Igh^a)	$CB6F_1$ anti-Meth A	1800	200	1400	200
Balb/c (Igh^a)	C.B20 anti-Meth A	1500	300	1500	100
$CB6F_1$ (Igh$^{a/b}$)	–	6000	1200	4900	5400
$CB6F_1$ (Igh$^{a/b}$)	Balb/c anti-Meth A	6700	1000	5700	5000
$CB6F_1$ (Igh$^{a/b}$)	$CB6F_1$ anti-Meth A	8000	1500	4600	2400
$CB6F_1$ (Igh$^{a/b}$)	C.B20 anti-Meth A	7200	1100	4100	2800
C.B20 (Igh^b)	–	3100	1100	2300	2700
C.B20 (Igh^b)	Balb/c anti-Meth A	2900	900	2100	2600
C.B20 (Igh^b)	$CB6F_1$ anti-Meth A	3000	1100	2400	2400
C.B20 (Igh^b)	C.B20 anti-Meth A	3500	1000	2200	2800

[a] 10^7 unprimed spleen cells were stimulated in primary anti-SRBC cultures for 5 days. The Igh haplotypes of the spleen cells are given in parentheses

[b] Antisera were added at a final concentration of 1% on day 0 of culture. Cultures marked with – indicate cultures of spleen cells only; T_s indicates cultures of spleen cells $+2\times10^5$ syngeneic T suppressor cells: $T_s+T_{cs}F$ indicates cultures of spleen cells, syngeneic suppressor cells at 2×10^5, and T contrasuppressor factor added at a final dilution of 10% on day 0 of culture; and T_s+T_{cs} indicates cultures of spleen cells, T suppressor cells at 2×10^5, and syngeneic T contrasuppressor cells at 2×10^5

the Meth A antigen [3]. When antisera was absorbed with tumor cells passed in either Balb/c or $CB6F_1$ mice, only F_1 passed tumor cells absorbed the activity (Table 2), suggesting a higher density of relevant antigen on cells passaged in F_1 mice. When Balb/c, $CB6F_1$, or C.B20 tumor-bearing mice were assayed for metastasis, in repeated experiments less than 15% of Balb/c or C.B20 mice had lymph node metastasis, while greater than 93% of $CB6F_1$ mice developed metastasis after injection of Meth A cells.

D. Discussion

An additional activity, the blocking of contrasuppression, has been found in antisera

Table 2. Tumors passed in $CB6F_1$ but not Balb/c absorb blocking activity

Assay cells[a]	Absorbing cells[b] passed in:	Anti-SRBC PFC/culture[c]		
		–	T_s	T_s+T_{cs}
Balb/c	No sera added	1600	300	1200
Balb/c	Sera not absorbed	1600	300	500
Balb/c	Balb/c	1700	300	700
Balb/c	$CB6F_1$	1500	200	1300
$CB6F_1$	No sera added	7000	2000	5700
$CB6F_1$	Sera not absorbed	8000	2100	2800
$CB6F_1$	Balb/c	7400	1800	3100
$CB6F_1$	$CB6F_1$	7400	2100	5900

[a] See footnote [a], Table 1

[b] Meth A cells passed in either Balb/c or $CB6F_1$ mice were used to do a double absorbtion of the antisera as described [1]

[c] See footnote [b], Table 1

against MC-induced tumors. Experiments with F_1 and *Igh* congenic mice indicate that effective antisera can only be generated in mice containing *Igh* disparate genes, while activity is only directed against cells expressing the Igh^a gene locus. This brings up the apparent dichotomy that F_1 mice generate autoantibody to their own *Igh*-linked gene products. However, tumors passaged in F_1 animals express the relevant antigen in a much higher surface density than does the parental strain. This "adaptive differentiation" process may explain the difference in tumorgenicity between F_1 and Balb/c mice, as measured by metastasis. The intriguing possibility exists that F_1 mice produce autoantibodies that block the generation of their own contrasuppressor cells, and that these contrasuppressor cells are important in controlling tumor metastasis. It also suggests that while many tumor cells escape immune destruction by generating suppressor T cells to depress immune responses, malignant cells may also escape by "encouraging" immunity, e.g., generating antigens which mimic normal cellular interaction structures and thereby blocking important cellular communication mechanisms needed to generate effective antitumor immunity.

References

1. DeLeo et al. (1977) J Exp Med 146:720
2. DeLeo et al. (1979) Proc Natl Acad Sci USA 76:2420
3. Flood et al. (to be published) Proc Natl Acad Sci USA
4. Green et al. (1981) Eur J Immunol 11:973
5. Janeway et al. (1975) Nature 253:544
6. Yamauchi et al. (1981) J Exp Med 153:1547