Short Communication

Assignment of the gene encoding for Meth A tumour rejection antigen (TATA) to Chromosome 12 of the mouse

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Tumours induced in the mouse by chemical carcinogens usually express specific antigens of the transplantation type, TATA. These antigens are demonstrated by immunizing syngeneic hosts with cells, purified membranes, soluble or solubilized antigen preparations (DuBois et al., 1980; DuBois et al., 1982; Rogers and Law, 1981; Ransom et al., 1981; Natori et al., 1981; Pellis and Kahan, 1975) and challenging with the same tumour. The usual finding is that these TATAs are highly immunogenic, stable and heritable. An intriguing feature of these chemically induced tumours is their uniqueness, each bearing a distinct antigen with an absolute restriction to the tumour of origin.

Recent investigations have focused upon defining the nature of these tumour specific antigens (TSA) and the nature of the genes coding for TSA. Several TATA have now been isolated and purified to chemical homogeneity (DuBois et al., 1982). Also, somatic cell genetic techniques have been employed in order to define the genetic basis of antigen diversity (Pravtcheva et al., 1981). This latter technique has been used in studies of attempts to assign the gene coding for the individually distinct Meth A antigen to a particular chromosome. This study was facilitated by the availability of a serologic assay to detect the Meth A antigen. De Leo et al. (1977) have recently produced a syngeneic antiserum that defined a cell surface antigen, TSSA, highly restricted to the methylcholanthrene-induced sarcoma, Meth A. There now exists good evidence that the serologically defined Meth A antigen TSSA is closely related to the individually distinct transplantation antigen (TATA) expressed by Meth A (DuBois et al., 1980; DuBois et al., 1981; DeLeo et al., 1982).

Pravtcheva et al. (1981) using somatic cell hybrids obtained by fusing cells of Meth A and E-36, an established cell line from Chinese hamster lung cells, and the typing antisera produced from Meth A immunizations, were able to assign the Meth A antigen, TSSA, to mouse chromosome 12 close to the genetic determinants for the Ig heavy chain. The present study extends these findings to show a clear correlation between chromosome 12 and Meth A antigen expression as detected by in vivo tumour rejection assays.

BALB/c female mice, 10–12 weeks of age, were immunized 3 times, 7 days apart with the somatic hybrid cells and control cells listed in Table I with a (5), then challenged 7 days after the last immunization with Meth A sarcoma. Typical results are shown in Figure 1. Protection against Meth A challenge was achieved with those somatic cell hybrids mAE 28 and ma 8c, groups 1–3, bearing the X<sup>12</sup> chromosome (chromosome 12 translocated to the X chromosome, and expressing the Meth A antigen as determined in a previous study by Pravtcheva et al., 1981 in the serologic assay);

| Hybrids/controls | Mouse chromosome present<sup>a</sup> | Meth A antigen (TSSA) expression<sup>b</sup> |
|------------------|------------------------------------|---------------------------------------------|
| mAE 28           | X<sup>12</sup>                      | +<sup>e</sup>                               |
| ma 8c            | X<sup>12</sup>                      | +<sup>e</sup>                               |
| ma 12            | X<sup>12</sup>                      | +                                           |
| mAE 6            | X<sup>12</sup>                      | +                                           |
| ms 5             | X                                  | −<sup>e</sup>                               |
| mAE 19           | 1,X                                | −<sup>e</sup>                               |
| Meth A           | X<sup>12</sup>                      | +<sup>e</sup>                               |
| E 36             | −                                   | −<sup>e</sup>                               |

<sup>a</sup>Chromosomes were identified by karyotype and isozyme analyses. (Pravtcheva et al., 1981).

<sup>b</sup>Meth A antigen expression determined by absorption of anti-Meth A cytotoxic activity from antisera; absorption (+) in all cases was comparable with absorption by Meth A cells. (Results adapted from Pravtcheva et al., 1981).

Somatic cell hybrids and control cells that were assayed for tumour rejection in the present study. Karyotyping and isozyme analyses were performed by Dr D. Pravtcheva. The cells were passaged once in tissue culture prior to being used in the tumour rejection assays.
Table

| Group Immunogen | % of control tumour volume |
|------------------|-----------------------------|
| 1 mAE 28         | P<0.001                     |
| 2 mAE 28         | P<0.01                      |
| 3 ma 8c          | P<0.01                      |
| 4 ms 5           |                             |
| 5 mAE 19         |                             |
| 6 THO            |                             |
| 7 MethA          |                             |
| 8 mAE 28         |                             |
| 9 mAE 28         |                             |

Figure 1 Inhibition of growth of Meth A sarcoma expressed as percentage of the control tumour volume in BALB/c recipients immunized with tissue culture grown cells of the several somatic cell hybrids between Meth A and E 36. Immunizations with $3 \times 10^6$ cells, 7 days apart x 3. Challenges with $2 \times 10^4$ Meth A cells, except in group 2 where $5 \times 10^4$ Meth A cells were used as challenge. Groups 8 and 9 are specificity controls; group 8 was challenged with sarcoma CI-4 and group 9 with sarcoma CII-10, $5 \times 10^5$ cells for each challenge tumour after appropriate immunizations with mAE 28 somatic cell hybrid.

In order to stabilize the several somatic cell hybrids and prevent loss of mouse chromosomes, these hybrid cells were hybridized with a clone of BALB/3T3-THO (see Pravtcheva et al., 1981). THO cells were therefore used as negative controls for immunization. See group 6.

hybrids ms 5 bearing the X chromosome and mAE 19 bearing chromosome 1 and X and neither expressing the Meth A TSSA (groups 4 and 5) did not prevent growth of Meth A sarcoma. Protection achieved with the hybrid cells bearing $X^{12}$ was similar to that achieved by immunization with control Meth A cells, the source of the $X^{12}$ chromosome. These results parallel exactly the results obtained in the serologic assays as shown in Table I, again showing the close relationship of TSSA and TATA in this system. Groups 8 and 9, Figure 1 are specificity assays. mAE 28-immunized BALB/c mice challenged with CI-4 and CII-10 respectively were not protected against challenge.

Each of these methylcholanthrene-induced sarcomas has its own strong TATA (Law, 1980).

Figure 2 shows typical growth patterns of the Meth A sarcoma in controls and in mice immunized with the somatic cell hybrids mAE 28 and ma 8C, bearing the $X^{12}$ chromosome.

As shown previously using serologic assays to detect the Meth A tumor specific antigen, only those somatic cell hybrids (Meth A x E 36) bearing the $X^{12}$ translocation were capable of immunizing BALB/c mice against Meth A challenge. Hybrids containing X as a single chromosome (ms 5) or X accompanied by chromosome 1 (mAE 19) did not effectively immunize against Meth A antigen expression in the serologic assay. There is therefore a clear correlation of Meth A antigen (TSSA and TATA) expression and the presence of chromosome 12.

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