Experimental induction of testicular teratomas in dissociated–reaggregated chimaeric gonads

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SUMMARY

Testicular teratomas can be experimentally induced in some strains of mice by grafting 12.5-day male genital ridges to the testes of adults. The grafts develop into testes and most of them have teratomas. The cells of 12.5-day foetal gonads were dissociated and the germ cells and somatic cells were separated. When germ cells were reaggregated with somatic cells and implanted in adult testes, they formed seminiferous tubules with teratomas. The somatic cell populations were contaminated with about 1% germ cells, and when they were implanted in adult testes, they formed testes with a comparatively low incidence of teratomas. When germ cells of a highly susceptible strain were combined with somatic cells from a resistant strain, they formed chimaeric testes with a high incidence of teratomas. When germ cells from a resistant strain were combined with somatic cells from a susceptible strain they formed chimaeric gonads and the incidence of teratomas was low. This indicates that at 12.5 days the genotype of the germ cells is responsible for susceptibility. When germ cells from older foetal gonads were combined with somatic cells of 12.5-day gonads, the incidence of teratomas was low. This showed that 12.5-day somatic cells cannot 'rejuvenate' older germ cells in a way to regain their susceptibility. When 12.5-day germ cells of highly susceptible strains were combined with older somatic cells the incidence of tumours was low indicating that the age of the somatic cells influences susceptibility to teratocarcinogenesis.

INTRODUCTION

Testicular teratomas (for definition see Stevens & Pierce, 1975a) can be induced in some inbred strains of mice and hybrids by grafting foetal male genital ridges to the testes of adults (Stevens, 1964). They develop into testes and most have teratomas. The genetic background of the donor strain was important. Grafted ridges from inbred strains 129 and A/He or their F1 hybrids showed a high incidence of tumour formation (Stevens, 1970a), whereas ridges from other strains showed a low susceptibility to tumour formation (Stevens, 1975b). Recently, Stevens (1981) has produced recombinant inbred lines between 129

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and A/He. When genital ridges were grafted to the adult testes, some lines showed an incidence of teratomas higher than either parent strain, whereas in other lines the tumour incidence was intermediate or low. This finding further demonstrates a genetic basis for the susceptibility to experimental induction of testicular teratomas.

The age of the graft is also important. Ridges from 11- to 13-day foetuses are highly susceptible to teratocarcinogenesis. The susceptibility is low at day 14 and extremely low or lost in foetal gonads obtained from embryos older than 14 days (Stevens, 1966, 1970a).

In early stages, testicular teratomas are located within the seminiferous tubules (Stevens, 1962, 1964). Stevens (1967) grafted genital ridges of homozygous Steel embryos to the testes of adults. These embryos have few if any primordial germ cells (PGC). Teratomas were obtained in none of the grafts, whereas 75% of the grafts from littermates with a normal number of PGC had teratomas. This finding supports the hypothesis that testicular teratomas originate from PGC.

The mechanisms underlying the initiation of tumorous growth in PGC to form teratomas are poorly understood (Solter, Adams, Damjanov & Koprowski, 1975). Evidence has been presented that temperature is involved in the experimental induction of testicular teratomas (Stevens, 1970b). Recently, a prolonged mitotic activity was found to be directly associated with spontaneous testicular teratocarcinogenesis (Noguchi & Stevens, 1982). Little is known about the control of divisions in PGC (Eddy, Clark, Gong & Fenderson, 1981).

The somatic environment can influence cell division in PGC. The initiation of meiosis of female PGC is induced by the somatic environment and is not autonomous (Byskov, 1974; Byskov & Saxen, 1976). Male germ cells enter a state of mitotic quiescence at 15 days of gestation. Shortly after birth the cells make contact with the basement membrane of the seminiferous cords and resume mitotic activity. It has been assumed that contact with the basement membrane reinitiates cell division (Nebel, Amerose & Hackett, 1961). The proliferative activity of PGC might also be controlled by a mitogenic polypeptide found in prepuberal Sertoli cells (Feig, Bellvé, Horback Erickson & Klagsbrun, 1980).

In order to analyse the influence of somatic environments on experimental induction of teratomas, chimaeric gonads were produced in vitro by means of dissociation–reaggregation experiments. Gonads dissociated into single cells can reorganize in vitro into male or female structures (Ohno, Nagai & Ciccarese, 1978; Zenzes, Wolf, Guenthner & Engel, 1978; O & Baker, 1978). Methods of separating germ cells from somatic cells have been developed (Steinberger & Steinberger, 1966; Yamada, Yasue & Matsumoto, 1973). We recombined germ cells from genital ridges with different susceptibilities to teratocarcinogenesis with somatic cells of different origins and grafted the reaggregates to the testes of adults to determine their ability to form teratomas. This report shows that dissociation–reaggregation experiments provide a useful tool to analyse some of the factors underlying testicular teratocarcinogenesis. The genotype of the
PGC population is crucial. Evidence is also presented that, depending upon the age of the foetal gonad, the somatic environment can influence the occurrence of the neoplastic change.

**MATERIALS AND METHODS**

*Animals and tumour incidences in genital ridge grafts*

The following strains of mice were used: 129/Sv-C P S1' (Stevens, 1970a) referred to as 129 in this report, A/HeJ, and C57BL/6J (BL/6) obtained from the Animal Resources Department of the Jackson Laboratory, and two recombinant inbred lines, whose progenitors were 129 and A/He: 129XA-F, 129XA-M (Stevens, 1981). They will be referred to as XF and XM, and are in the 18th generation of brother/sister matings. (129 x A/He)F1 hybrids will be referred to as F1.

When the genital ridges were grafted to testes of adults, the following tumour incidences were obtained: (129 x A/He)F1 97% (12-day), 85% (13-day), 10% (14-day), 0% (15-day) (data from Stevens, 1970a); XM 12-day: 95% (37 grafts with tumours/39 ridges grafted), 13-day: 85% (53/62), 14-day: 6% (1/16), XF 12-day: 4% (1/26), 13-day: 6% (1/16), C57BL/6J no tumours were obtained in eighteen 12- and 13-day ridges grafted. Most XM grafts contained many more tumour foci than F1 ridge grafts.

*Genital ridges and foetal gonads*

The ages of the foetuses were determined by examining females in the morning for the presence of a copulation plug. The day on which the plug was found was considered as day 0 of gestation. Genital ridges and foetal gonads were dissected from the embryos in the afternoon. Assuming that mating took place in the middle of the light–dark cycle, ridges dissected out on the thirteenth day of pregnancy were between 12.5 and 13 days old. At that age, the sex of the foetuses could be distinguished by examination of the foetal gonads.

*Isolation of germ cells and somatic cells*

The kidney was removed from the gonadal part of the genital ridge with tungsten needles. Male gonads of the same age were incubated for 10 min at 32 °C in 0.25% (w/v) trypsin (Difco) in Rinaldini's salt solution (Rinaldini, 1959). After the incubation period, the gonads were pipetted vigorously until cells were completely dissociated. The trypsin solution was diluted with an equal volume of Hanks balanced salt solution (Gibco) containing 10% (v/v) foetal bovine serum (FBS) (Gibco). The cells were centrifuged (~ 300 g) in Falcon polypropylene tubes in an International Microcentrifuge for 10 min and washed once with Hanks + 10% FBS. Somatic and germ cells were resuspended in medium NCTC 109 (Microbiological Associates) supplemented with 10% FBS, penicillin G (Sigma, 100 i.u./ml), streptomycin (Sigma, 0.1 mg/ml)
and incubated overnight at 32 °C in Falcon Petri dishes in a Torbal chamber (Torsion Balance Company, Clifton, New Jersey), gassed with 10% CO₂ balanced air. Germ cells and somatic cells were separated according to the method described by Steinberger and Steinberger (1966) and Yamada et al. (1973). Germ cells which did not stick to the plastic surface were pipetted off. The attached somatic cells were washed once with Hanks and detached with 0.025% (w/v) trypsin in Rinaldini’s solution. The trypsin solution was diluted with an equal volume of Hanks + FBS, the cells were pelleted and washed once in Hanks + 10% FBS. Both cell populations were stained for alkaline phosphatase (Sigma kit 85L-2) to identify the germ cells. The 12.5- and 15.5-day somatic cell populations contained 1% alkaline-phosphatase-positive germ cells, which could not be removed by the procedure used. The cell viability was 94% as determined by trypan-blue-dye-exclusion test. The 12.5- and 15.5-day germ cell population contained about 20% of alkaline-phosphatase-negative cells. The viability in the 12.5- and 15.5-day germ cell population was between 60 and 70%, and the enzyme-negative cells were most likely dead germ cells. A similar separation of enzyme-positive and enzyme-negative cells was obtained using 16.5- and 17.5-day foetal gonads. The following average cell numbers per genital ridge or foetal gonad were obtained: 12.5-day 4.1 x 10³ germ cells, 11.8 x 10³ somatic cells; 15.5-day, 17.1 x 10³ germ cells, 72.3 x 10³ somatic cells; 16.5-day, 17.5 x 10³ germ cells, 92.8 x 10³ somatic cells; 17.5-day, 23.9 x 10³ germ cells, 108.5 x 10³ somatic cells. These data were obtained from 10–20 pooled 12.5-day foetal gonads and from 6–8 foetal gonads of 15.5-day or older embryos. The cell number determined for 12.5-day genital ridges is in good agreement with other reports (Mintz & Russell, 1957; Tam & Snow, 1981).

**Aggregated gonads**

For a single experiment the cellular material of 8–12 (usually 10) foetal gonads was used. Germ cells and somatic cells were recombined, mixed and centrifuged in a Falcon polypropylene tube. The supernatant was removed until 200 to 300 µl were left over the pellet. The cells were resuspended and transferred into a conical 400 µl plastic tube, which was previously sterilized in 70% ethanol and centrifuged (~300 g) for 20 min. The pellet was transferred with a 20 µl micropipette (Microcaps) onto an agar-coated metal grid sitting in a Falcon Petri dish (Steinberger & Steinberger, 1971). The metal grid covered with 2.5% Agar–Noble (Difco) in 0.7% NaCl was previously placed in the Petri dish and equilibrated with culture medium. Cultures were placed in a Torbal chamber, gassed with 10% CO₂ in air and incubated at 32 °C for one day.

When 12.5-day germ or somatic cells were combined with 15- to 17-day somatic or germ cells, the ratio between germ cells and somatic cells was adjusted to the ratio found in a 12.5-day foetal gonad.

Aggregated gonads cultured for one day in vitro were tested for their susceptibility to teratocarcinogenesis by transplantating them to testes of F₁ adult
males unless otherwise stated. The testes were exposed by a ventral incision and the graft was inserted under the testis capsule using a micropipette.

Analysis of aggregates and grafts

Grafts were recovered after 10–15 days unless otherwise stated, fixed in Vandegrift's solution, embedded in paraffin and serially sectioned at 7 μm. Reaggregates were fixed in 3% glutaraldehyde/2% formaldehyde/0.1 M cacodylate buffer, pH 7.4 (2 h), rinsed twice (1 h) in 0.1 M cacodylate buffer, dehydrated in alcohol, embedded in JB-4 plastic resin (Polysciences Inc., Warrington, PA), and sectioned at 2 μm. Sections were stained with haematoxylin and eosin for histological analysis. Tumours were easily identified in histological sections using the criteria described by Stevens (1962). Numbers of tumour foci were estimated by examining serial sections. Low numbers could be counted accurately but high numbers were difficult to count, because individual foci overlapped and could no longer be distinguished.

Glucose phosphate isomerase-1 (GPI-1) analysis

Macroscopically visible tumours were dissected out 7 weeks after grafting the reaggregate to the testis. The tumour was freed from attached host testicular tissue and the GPI-1 pattern analysed according to Eicher & Washburn (1978).

RESULTS

(1) Teratoma formation in dissociated–reaggregated gonads

Germ cells and somatic cells were allowed to separate in culture overnight. The two cell populations were recombined and the cell pellet cultured for 1 day in vitro. The organ-like reaggregate contained apparently randomly mixed germ cells and somatic cells. When 12.5-day genital ridges were used as a starting material, many germ cells were in mitosis after the culture period (Fig. 1). The reaggregate was grafted to the testis of an adult mouse and after five days in vivo the first testicular tubules appeared (Fig. 2). The type-specific grouping of cells continued and after 10 days tubules were prominent (Fig. 3). After 13 days in vivo, the graft appeared very similar to a 12.5-day genital ridge grafted directly to the testis (Fig. 4). Similarly, when female gonads were dissociated and recombined, typical female structures were found in the transplanted reaggregate.

Testicular teratomas were obtained in the reaggregates, indicating that the somatic cells and the germ cells had retained their characteristics with respect to tumour formation, despite the experimental manipulations (Fig. 3, 4). Reaggregates from F1 12.5-day male foetal gonads formed tumours in 4 of 12 independent experiments. The tumour incidence increased to 93% when reaggregates of foetal gonads from the highly susceptible recombinant inbred line XM were used. No tumours were obtained with the recombinant inbred
Fig. 1. Germ cells and somatic cells of a 12.5-day male foetal gonad reaggregated and cultured for 1 day in vitro. Some germ cells are in mitosis. JB-4 section, × 500.

Fig. 2. Germ cells and somatic cells of 17-day male foetal gonads reaggregated, cultured for one day in vitro and transplanted to an adult testis for 5 days. Testicular tubules have started to form (arrows), × 130.
Experimental induction of testicular teratomas

In order to study the crucial role of germ cells in testicular teratocarcinogenesis, we reaggregated the somatic cell populations without adding PGC. These formed tubular structures, as did the germ-cell-containing reaggregates. Tumours were obtained in 46% of the reaggregates, compared to the 93% when the total germ cell number was present (Table 1). In a normal 12.5-day foetal gonad about 25% of cells are PGC. The somatic cell population used for reaggregation was contaminated with about 1% germ cells. The tumour frequency in germ-cell-deficient gonads was unexpectedly high, and indicates that only a few germ cells are required for the neoplastic change.

The low number of primordial germ cells present was reflected in the number of tumour foci per graft. In germ-cell-deficient reaggregates the number of foci was considerably smaller (two to four) than in aggregates with a normal number of germ cells (eight to too numerous to count) (Table 1). Both the decrease in tumour frequency and number of foci per reaggregate demonstrate the importance of primordial germ cells in testicular teratocarcinogenesis.

(2) Teratotermata formation in chimaeric gonads

(A) Isochronic combinations between strains with low and high susceptibilities

Experimental teratomas can be induced in foetal gonads of some strains of mice at only a low or zero frequency (Stevens, 1975b). It is unknown whether this is due to the genetic background of the germ cells alone or whether the somatic environment modulates the susceptibility to experimental induction of teratomas.

XM (high incidence) germ cells from 12.5-day foetal gonads were combined with somatic cells of foetal gonads from the recombinant inbred line XF (low incidence) of the same age. In 88% of aggregates of XM germ cells and XF somatic cells numerous tumour foci were obtained (Table 2). In reciprocal experiments, XF germ cells recombined with XM somatic cells, the tumour incidence decreased to 45% and the number of foci was low (Table 2). The frequency and the number of foci was similar to the finding in germ-cell-deficient XM reaggregates (Table 1). No evidence was found that the susceptibility of XF germ cells could be changed by the XM somatic cell environment. This was further demonstrated in the following experiments. Tumours were obtained when XM germ cells were recombined with F₁ somatic cells (2/3), but XF germ-cell populations did not form tumours (0/4) in this environment (Table 2).

These results show that the susceptibility to teratocarcinogenesis in 12.5-day genital ridges is determined by the genotype of the PGC. The somatic cells of genital ridges from strains with low and high susceptibilities do not effect the neoplastic change in isochronic recombinations. Apparently, the degree of
susceptibility to experimental teratocarcinogenesis in a 12.5-day genital ridge is a germ-cell-autonomous trait.

To identify the origin of the tumours, the above-mentioned experiments were repeated using strain BL/6 instead of XF. BL/6 is homozygous for the allozyme GPI-1B, whereas 129 and A/He are homozygous for GPI-1A. Aggregates were grafted to the testes of (BL/6 × XM)F<sub>1</sub> adult males for 7 weeks. Macroscopically visible tumours were prepared for enzyme analysis after a small part of the tumour had been removed for histological examination. Other grafts which did not grow significantly were analysed histologically.

In three experiments XM germ cells were combined with BL/6 somatic cells. One aggregate grew to a macroscopically visible teratoma, a second aggregate formed a smaller tumour detected histologically. The tumour expressed predominantly the XM allozyme GPI-1A, indicating that it must have arisen from the XM PGC population. In three reciprocal combinations, BL/6 germ cells combined with XM somatic cells, two macroscopically visible teratomas were obtained expressing also the XM allozyme of GPI-1 (Fig. 5). This indicates that the tumours must have been derived from cells of the XM somatic cell population (which contained a few XM germ cells) and not from the BL/6 PGC. Since the somatic cells were never totally pure and contained a few PGC, the tumours most likely originated from these germ cells. These results show again that the PGC from the low-susceptibility strain BL/6 cannot be activated to become highly teratocarcinogenic by the somatic environment of a strain with a high susceptibility to teratocarcinogenesis. The fact that in both series of experiments tumours expressed only the XM allozyme of GPI-1 is in agreement with the interpretation of previous results (Stevens, 1967), that testicular teratomas originate from PGC.

(B) Combinations between gonads of different developmental stages (heterochronic chimaeric gonads)

The susceptibility to experimental teratocarcinogenesis is extremely low in foetal gonads from foetuses older than 14 days (Stevens, 1966, 1970a). The germ cells have either matured to a stage where they are no longer susceptible or the somatic environment is no longer permissive to allow the neoplastic change to occur.

Germ cells from susceptible F<sub>1</sub> or XM 12.5-day foetal gonads were combined with somatic cells isolated from gonads of 15- to 17-day foetuses. Tumours were

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Fig. 3. Reaggregate of germ and somatic cells from 12.5-day male foetal gonads transplanted to an adult testis for 10 days. Note testicular tubules and tumour foci (arrows), × 150.

Fig. 4. Reaggregate of germ and somatic cells from 12.5-day male foetal gonads transplanted to an adult testis for 13 days. Note testicular tubules and tumour foci (arrows), × 130.
Experimental induction of testicular teratomas
Table 1. *Teratoma formation is dissociated-reaggregated gonads*

<table>
<thead>
<tr>
<th>Source of genital ridges</th>
<th>Germ cells</th>
<th>Somatic cells</th>
<th>No. reaggregates</th>
<th>Reaggregates with tumours</th>
<th>With tumours (%)</th>
<th>Foci no. per reaggregate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>129XA-M</td>
<td>+</td>
<td>+</td>
<td>14</td>
<td>13</td>
<td>93</td>
<td>8–TNTC†</td>
</tr>
<tr>
<td>(129 × A/He)F₁</td>
<td>+</td>
<td>+</td>
<td>12</td>
<td>4</td>
<td>33</td>
<td>3–TNTC‡</td>
</tr>
<tr>
<td>129XA-M</td>
<td>−</td>
<td>+</td>
<td>13</td>
<td>6</td>
<td>46</td>
<td>(2–4)</td>
</tr>
<tr>
<td>129XA–F</td>
<td>+</td>
<td>+</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>129XA–M</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

* TNTC = too numerous to count.  † Most abundant foci numbers. ‡ Gives total range.

Table 2. *Isochronic chimaeric gonads between strains with low and high susceptibilities to teratocarcinogenesis*

<table>
<thead>
<tr>
<th>Germ cells</th>
<th>Somatic cells</th>
<th>No. reaggregates</th>
<th>Reaggregates with tumours</th>
<th>With tumours (%)</th>
<th>Foci no. per reaggregate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>129XA–M</td>
<td>129XA–F</td>
<td>8</td>
<td>7</td>
<td>88</td>
<td>4–TNTC†</td>
</tr>
<tr>
<td>129XA–F</td>
<td>129XAM</td>
<td>11</td>
<td>5</td>
<td>45</td>
<td>1–3</td>
</tr>
<tr>
<td>129XA–F</td>
<td>(129 × A/He)F₁</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1–9</td>
</tr>
<tr>
<td>129XA–M</td>
<td>(129 × A/He)F₁</td>
<td>3</td>
<td>2</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td>(129 × A/He)F₁</td>
<td>129XA–F</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>~5</td>
</tr>
</tbody>
</table>

* TNTC = too numerous to count. † Gives most abundant foci numbers. ‡ Gives total range.

obtained in only 17% (2/12) of the aggregates, whereas in the internal control, where 12.5-day germ cells were reaggregated with somatic cells of the same age, tumours were recovered in 92% of the reaggregates (Table 3). Both tumours in the chimaer gonads occurred in seven aggregates where 15-day somatic cells were used.

In the same series of experiments, 12.5-day somatic cells were aggregated with the germ cells from foetuses of 15- to 17 days of age. Tumours were recovered in 33% of the transplanted aggregates (Table 3). Three of the tumours occurred in eight experiments where XM somatic cells were used; one tumour was obtained in four experiments using F₁ somatic cells. These are tumour frequencies expect-
Experimental induction of testicular teratomas

Fig. 5. GPI-1 analysis of macroscopically grown tumours. Slot (1), C57BL/6J testis; (2) 129XA-M testis; (3) (C57BL/6J × 129XA-M)F₁ testis; (4)–(6) tumours, 129XA-M germ cells/C57BL/6J somatic cells (4); C57BL/6J germ cells/129XA-M somatic cells (5, 6).

Table 3. Heterochronic chimaeric gonads between foetal gonads of different developmental stages

<table>
<thead>
<tr>
<th>Germ cells (age in days of gestation)</th>
<th>Somatic cells (age in days of gestation)</th>
<th>No. reaggregates</th>
<th>Reaggregates with teratomas</th>
<th>With teratomas (%)</th>
<th>Foci no. per reaggregate</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-5</td>
<td>12-5</td>
<td>12†</td>
<td>11</td>
<td>92</td>
<td>8–TNTC¶ (1–TNTC)**</td>
</tr>
<tr>
<td>12-5</td>
<td>15–17</td>
<td>12‡</td>
<td>2</td>
<td>17</td>
<td>1–2</td>
</tr>
<tr>
<td>15–17</td>
<td>12-5</td>
<td>12§</td>
<td>4</td>
<td>33</td>
<td>1–3</td>
</tr>
<tr>
<td>12-5</td>
<td>15–17/12-5 (1/1 ratio)</td>
<td>4</td>
<td></td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

* TNTC = too numerous to count.
‡ Gives most abundant foci numbers.
** Gives total range.
† Nine experiments with 129XA-M, 3 experiments with F₁.
‡ Eight experiments with 129XA-M, 4 experiments with F₁.
§ Eight experiments with 129XA-M, 4 experiments with F₁.
|| All experiments with 129XA-M.

ed when germ-cell-deficient somatic cells were reaggregated (Table 1). The number of tumour foci was small in these aggregates compared to the numbers in the control experiments. No evidence was obtained that germ cells from gonads which have lost the susceptibility to teratocarcinogenesis can be reactivated to form tumours in a 12-5-day somatic environment.

The results indicate that primordial germ cells undergo a stage of maturation and lose the susceptibility to teratocarcinogenesis in the course of foetal development. Furthermore, at the time these cells have lost the susceptibility, the somatic cell population has differentiated to a stage where it allows the neoplastic
change to occur only rarely if ever. The importance of the state of differentiation of the somatic elements is indicated by the fact that tumours can form when 12.5-day germ cells were combined with a somatic environment consisting of equal numbers of 12.5- and 15- to 17-day somatic cells (Table 3).

**DISCUSSION**

Cell-type-specific grouping (Moscona, 1956) leading to the formation of testes and ovaries was obtained after grafting dissociated-reaggregated cells of foetal gonads to the testes of adults. This has also been observed in reaggregates cultured *in vitro* (O & Baker, 1978; Regenass, unpublished). The germ cell population survived the culture period and retained their ability to form teratomas. Tumour formation was found to be dependent upon the presence of PGC in agreement with a germ cell origin of testicular teratomas (Stevens, 1967). Tumour formation in isochronic and heterochronic (Moscona, 1956) chimaeric gonads produced *in vitro* lead to the conclusion that the degree of genetic susceptibility of the PGC and the age of the male somatic environment are crucial in experimental testicular teratocarcinogenesis. This is based on the findings that (1) tumour formation is an autonomous trait of the germ cell fraction in a 12.5-day male somatic environment, (2) germ cells from foetal gonads which have lost the susceptibility to teratocarcinogenesis in the course of development could not be reactivated to form tumours in a 12.5-day somatic environment, and (3) at the time foetal gonads have lost the susceptibility in the course of development, the somatic elements of the gonads have matured to a stage where they no longer promote the neoplastic change.

The tumour susceptibility in grafted reaggregates differed between the two highly susceptible foetal gonad donors, XM and F1 (Table 1). Grafts of XM and F1 genital ridges resulted in similar incidences, but the number of tumour foci per graft was often higher in XM (Stevens, 1981). When 12.5-day genital ridges were cultured *in vitro* for two days at 32 °C, the tumour incidence decreased to 21% for F1 ridges whereas the incidence remained high (82%) after a 3-day culture of XM ridges (Friedrich, Regenass, & Stevens, 1982). These differences might account for the tumour frequencies obtained in the reaggregates.

The incidence of tumours and the number of foci in the reaggregates were dependent upon the number of susceptible PGC present. In experiments where XM somatic cells alone were reaggregated (with few PGC), the tumour incidence decreased from 93% to 46% (Table 1). Similar frequencies and numbers of tumour foci were obtained when XM somatic cells were combined with germ cells of strains with a low susceptibility (XF) (Table 2), or with germ cells from 15- to 17-day gonads (Table 3). The strain of origin of the tumours was determined using a GPI difference between XM and BL/6, a strain with a low or zero susceptibility to teratocarcinogenesis. In both types of aggregates, XM germ cells and BL/6 somatic cells, BL/6 germ cells and XM somatic cells, the
tumours expressed predominantly the XM allozyme (Fig. 5). This finding is in agreement with a germ cell origin of teratomas, and indicates that those formed in the latter recombination most likely derived from germ cells contaminating the somatic cell population and not from the BL/6 germ cells. The germ cells remaining in the somatic cell population after the separation procedure were estimated to be about 1% of the total number. The high tumour incidence obtained in germ-cell-deficient reaggregates indicates that large numbers of germ cells are not required for teratoma formation. This finding is interesting with respect to the recent observation by Noguchi & Stevens (1982) that the seminiferous cords of strain 129/Sv-ter mice with bilateral spontaneous testicular teratomas contained only a few primordial germ cells, often associated with a prolonged mitotic activity. If mitotically active germ cells are a prerequisite for teratocarcinogenesis, compensatory proliferation in germ-cell-depleted reaggregates could account for the high tumour incidence.

Reaggregated gonads from the recombinant inbred line XF did not yield tumours. Since the male somatic cells of these 12-5-day gonads allowed the neoplastic change to occur at a high rate when combined with germ cells isolated from XM foetal gonads, the problem of tumour growth from somatic cell populations of highly susceptible strains can be solved in future experiments by using somatic cells of a strain with a low susceptibility as a permissive environment.

Germ cells from strains with a low susceptibility, and germ cells from foetal gonads which have lost the susceptibility to teratocarcinogenesis in the course of development, were combined with 12-5-day somatic cells of highly susceptible strains (F₁ and XM) in an attempt to activate them to produce teratomas. No evidence was obtained that these germ cells could respond to possible factors in 12-5-day somatic cells causing the neoplastic change. The finding that susceptible cells formed tumours in a 12-5-day somatic environment of a resistant strain indicates that tumour formation in a 12-5-day genital ridge is a germ-cell-autonomous trait. As the foetal gonad develops, the tumour cell progenitors become resistant to teratocarcinogenesis and are no longer able to undergo the neoplastic change.

The 12-5-day germ cells of a highly susceptible strain combined with the somatic elements of gonads which have lost their susceptibility in the course of development, formed tumours in only 17% of the aggregates. These experiments demonstrate the importance of the somatic environment in testicular teratocarcinogenesis. Both tumours were obtained in seven experiments using somatic cells from 15-day (rather than 17-day) foetal gonads. The fact that a low tumour incidence was obtained in these aggregates is not surprising, since a few experimentally induced teratomas were recovered after grafting gonads from 15-day (but not older) foetuses (Stevens, 1970a).

In experiments where somatic cells of susceptible and non-susceptible foetal gonads were mixed, tumours were recovered in all experiments. How the somatic cells influence the germ cells remains to be analysed.
The germ cells stop dividing at 14 days of gestation. At day 15 the PGC, previously mixed with the supporting cells, move to the centre of the seminiferous cords, whereas the supporting cells line up at the periphery (Peters, 1970). Evidence has been presented that the divisional activity of primordial germ cells is controlled by the somatic environment (Byskov, 1974; Nebel et al. 1961; Stein & Anderson, 1981). If the somatic environment also influences the mitotic activity in 14-day male foetal gonads, the fact that a very low tumour incidence was found when susceptible germ cells were combined with somatic cells of 15- to 17-day foetal gonads could be explained by a termination in mitotic activity. The nature of the action of the male somatic cells on susceptible PGC remains to be elucidated. Such a study might lead to a better understanding of the mechanism of testicular teratocarcinogenesis.

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Experimental induction of testicular teratomas


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