Sex chromosome chimaerism and the transmission of blood group genes by tetraparental rams

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Summary. A previously published study of 4 rams, 3 of them tetraparental chimaeras, has been extended. All were born subsequent to transplantation of separated blastomeres into intact 4-8-cell embryos which were transferred to recipient ewes. All were phenotypically normal males. Study of blood lymphocytes and red cell antigens showed that two were XX/XY chimaeras, one an XY/XY chimaera. Chimaerism was not identified in the 4th animal. The new data show that the previously reported decline in proportions of red cells derived from the one cell line has continued, although the proportions of lymphocytes entering mitosis in culture have remained nearly constant. A decline of potassium concentration in the red cells of one animal and of transferrin type in another also continued.

Analysis of blood types in the offspring of the 4 rams revealed that both cell lines of the XY/XY chimaera must have produced functional spermatozoa whereas the genes transmitted by the remaining 3 rams gave evidence of spermatozoa from one cell line only.

Introduction

Tucker, Moor & Rowson (1974) described three tetraparental sheep chimaeras produced by transplanting blastomeres into other embryos of different parental origin. The three sheep thus produced were phenotypically normal males although two of them were sex chromosome chimaeras having female cells present. Each ram showed a mixture of blood types which could only have been derived from both sets of parental genes, and it was concluded that these sheep were tissue chimaeras which had cells of both blastomere and egg donor origin. In a fourth ram in this series chimaerism was not identified and this animal apparently possessed blood group genes derived entirely from the transplanted blastomere cells. The main objective of the present investigation was to determine by breeding experiments whether genes of blastomere and/or egg donor origin were transmitted by these rams to their offspring. It was known by analogy with work on tetraparental mice (Mintz, 1968; Mullen & Whitten, 1971) that the ram which was an XY/XY chimaera could transmit both types of parental genes to his offspring, whereas the XX/XY chimaeras would only pass on genes derived from the male population of cells. In the initial studies of these sheep chimaeras it was noticed that the relative proportions of the two populations of red cells were changing with time. The present paper extends this study to show that the changes continued over a period of 5 years.

Materials and methods

Sheep

Blastomeres from embryos at the 4-8-cell stage of development derived from the mating of Finnish Landrace ram (E34) with a Welsh Mountain ewe (W952) were transplanted into embryos of the same age derived from the mating of a Suffolk ram (517) with another Welsh Mountain ewe (W827). The injected eggs were transferred to host mothers and the resulting offspring (Nos 8, 44, 47 and 46) were the rams used in the present study. Further details have been given in the previous paper (Tucker et al., 1974).
Serology and electrophoresis

The methods used for blood-typing the sheep have been described previously (Tucker et al., 1974). Determinations of the relative proportions of the two red cell populations in a chimaera were made by differential haemolysis using specific blood-typing reagents (Dain & Tucker, 1970).

Karyotyping

Somatic organ cultures. For lymphocytes, blood samples were taken from the jugular vein into sterile, heparinized syringes. Aliquots (1 ml) of whole blood were set up in RPMI 1640 culture medium (Flow Laboratories, Irvine, Scotland) with 25% fetal bovine serum (Flow Laboratories) and 1% phytohaemagglutinin (Wellcome, Beckenham, England) by a modification of the method of Basrur & Gilman (1964). Cultures of skin and kidney cells were prepared from fresh tissue by the following method of Dr M. Daniel (personal communication).

Approximately 15 g tissue were chopped into pieces of about 3 mm³, suspended in 25 ml 0·5% trypsin in phosphate-buffered saline, pH 7·4, containing 50 units penicillin/ml and 50 μg streptomycin/ml (Crystamycin: Glaxo, Greenford, England) and 2·5 μg Fungizone/ml (E.R. Squib and Sons, Inc. New York, U.S.A.) and shaken at 38°C for 1 h. The supernatant was discarded and the process repeated with 25 ml fresh trypsin solution. The second supernatant was discarded and the pieces shaken vigorously for 10 min, by hand, in 10 ml culture medium (90% RPMI 1640, 10% fetal bovine serum). The suspension was centrifuged at low speed for 5 min. The pellet of cells was washed once in medium, resuspended in 20 ml medium and dispensed into 4 × 4 oz glass medicine bottles. The cultures were incubated for 24 h at 38°C, and then the medium and unattached cells were poured off and fresh medium was substituted. The cells were 'cold-shocked' for 30 min at +4°C and 24 h later the cultures were treated for 15 min with Colcemid (15 μg/ml: Ciba Laboratories, Horsham, England) and detached with 0.5% trypsin in saline. Cells were treated for 10 min in hypotonic medium and fixed in acetic acid–methanol (1:3 v/v) by the standard method before being spread on ice-cold glass slides and stained with Giems.

Meiotic cells from testis. Rams 44 and 47 were anaesthetized with sodium pentobarbitone (Nembutal: Abbott Laboratories, Queenborough, Kent) and halothane (Fluothane: I.C.I. Pharmaceuticals Ltd, Macclesfield, Cheshire) before slaughter. A piece of tissue approximately 1 cm³ was taken from a testis of the anaesthetized ram and chopped quickly in hypotonic medium (20% Dulbecco's phosphate-buffered saline, pH 7·4:70% sterile distilled water:10% aqueous Colcemid solution (0·3 μg/ml)). The cell suspension was pipetted off and incubated at 28–31°C for 20 min. After centrifugation at low speed the pellet was resuspended in the acetic acid–methanol fixative. Three further changes of fixative were made before the suspension of cells was spread on ice-cold glass slides and air dried. The slides were stained with Giemsa.

Tissue from the normal ram was taken immediately after death at the slaughterhouse and processed in the same way.

Results

Blood types

The blood types of the two sets of parents giving rise to the rams used in the present study are shown in Table 1. The informative blood types of the blastomere donor (b line) parents were albumin type W, transferrin type D and red cell antigens K and N', all of which were absent in the egg donor (e line) parents. The latter had transferrin B and antigens A, B' and R not found in the b line. It was the presence of these diagnostic factors that had made it possible to trace the parentage involved in the rams (Tucker et al., 1974). Three of the rams (Nos 8, 44 and 47) had combinations of these diagnostic factors which showed they were chimaeras, each ram having two populations of red cells, or two transferrin or albumin phenotypes, one of blastomere and the other of egg donor origin. The fourth ram (No. 46) had a blood type ascribable only to that of the b-line parents and showed no evidence of chimaerism.
Table 1. Blood types of blastomere and egg donor parents of the chimaeric rams

<table>
<thead>
<tr>
<th>Blastomere donor (b line)</th>
<th>Egg donor (e line)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dam (W952)</strong></td>
<td><strong>Sire (E34)</strong></td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td><strong>S S</strong></td>
</tr>
<tr>
<td>Transferrin</td>
<td><strong>W W</strong></td>
</tr>
<tr>
<td>Red cell antigens</td>
<td><strong>A C</strong></td>
</tr>
<tr>
<td></td>
<td><strong>C D</strong></td>
</tr>
<tr>
<td></td>
<td><strong>K U N' O'x Cx M M' L O</strong></td>
</tr>
<tr>
<td></td>
<td><strong>U Cx M M' P R</strong></td>
</tr>
<tr>
<td></td>
<td><strong>A B' O'x Cx M M' L R</strong></td>
</tr>
<tr>
<td><strong>Dam (W827)</strong></td>
<td><strong>Sire (517)</strong></td>
</tr>
<tr>
<td></td>
<td><strong>S S</strong></td>
</tr>
<tr>
<td></td>
<td><strong>B C</strong></td>
</tr>
</tbody>
</table>

The types underlined are unique to the blastomere or egg donor.

These 4 rams were mated with ewes of known blood type and the resulting lambs were typed at weaning. Only those types which could not have been derived from the mother were considered. Table 2 gives the informative blood types of the rams and their offspring. The presence of a diagnostic factor in many of the lambs showed clearly the derivation of the types; in others it was possible to use additional factors (shown in parentheses in Table 2) as evidence of derivation because they were known to be present in only one red cell or transferrin line of the chimaeric ram. For example, in Ram 8, factors U O'x and P occurred in association with factors K and N' only in the b-line population and hence the appearance of any of these factors in the offspring would indicate b-line derivation.

Ram 8, an XX/XY chimaera, was mated to 6 ewes over two breeding seasons. In every case the blood type of his lambs was ascribable to the b line and there was no indication that any e-line genes had been transmitted. Ram 44, an XY/XY chimaera, was mated to 8 ewes over two breeding seasons and 8 of his 13 offspring could be ascribed to the e line and 3 to the b line. Two lambs were of a type which made it impossible to solve their origin. Ram 47, an XX/XY chimaera, was mated to 7 ewes over two breeding seasons and produced 17 lambs of which 15 were ascribable to the b line and two could not be solved. Ram 46 (XY) was not shown to be a chimaera, but of his 15 offspring from matings with 4 ewes over two breeding seasons 14 had a b-line blood type and one could not be solved.

Changes in blood type with age

Table 3 shows the relative percentages of the two populations of red cells in the rams over a period of 5 years. In the three chimaeras the b-line population decreased, and virtually disappeared in Rams 8 and 44. No change was observed in Ram 46 which always had 100% b-line cells. The b-line population in Ram 8 was high potassium (HK) type while the e-line cells were low potassium (LK) type (Tucker et al., 1974) and, as expected from the decline in the b line judged by antigen types (Table 3), the HK cells slowly disappeared, the ram eventually having a normal LK-type red cell potassium concentration (Table 4). The other rams showed virtually no change in their red cell potassium concentration and they were either HK or LK type with no evidence of admixture.

Rams 8 and 44 showed chimaerism of serum albumin types, i.e. SW plus SS. There was no evidence that the relative concentrations of these two types changed throughout the 5-year period. Ram 8 showed chimaerism of serum transferrin types AC and CC, and there was no change in their proportions as the ram aged. However, Ram 44, which at the outset had a transferrin chimaerism involving CC from the b line and AB from the e line, did show a change with age: transferrin C became weaker and weaker so that eventually only a normal AB type could be detected.

Karyotypes

In the previous paper (Tucker et al., 1974) it was shown that at 6 months of age Rams 8 and 47 had 89 and 34% 'female' lymphocytes respectively. The other two rams had no 'female' cells detectable. The karyotypes of cultured peripheral blood lymphocytes were determined on several occasions during the next 5 years. When the breeding experiments were completed, the three chimaeras were slaughtered. The macroscopic appearance of the testes and external male reproductive organs appeared to be normal: since the rams were fertile, the internal genitalia were not examined.
Table 2. Transmission of informative blood group genes to the offspring of the rams

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Informative blood types</th>
<th>Informative blood types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Albumin</td>
<td>Red cell antigens</td>
</tr>
<tr>
<td>Ram 8 (XX/XY)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>(i) K N' (U O'x P)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ii) A B' (L)</td>
</tr>
<tr>
<td>Offspring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>99, 23</td>
<td>W</td>
<td>K</td>
</tr>
<tr>
<td>89, 91</td>
<td>W</td>
<td>(P)</td>
</tr>
<tr>
<td>98</td>
<td>W</td>
<td>K (P)</td>
</tr>
<tr>
<td>13</td>
<td>W</td>
<td>K N' (U, P)</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>N'</td>
</tr>
<tr>
<td>93</td>
<td></td>
<td>b line</td>
</tr>
<tr>
<td>46</td>
<td></td>
<td>(P)</td>
</tr>
<tr>
<td>119</td>
<td></td>
<td>b line</td>
</tr>
<tr>
<td>Ram 47 (XX/XY)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>(i) N' (U O'x P)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ii) B' (?L)</td>
</tr>
<tr>
<td>Offspring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>94, 110, 112, 116</td>
<td>W</td>
<td>(P)</td>
</tr>
<tr>
<td>122</td>
<td>W</td>
<td>N' (U)</td>
</tr>
<tr>
<td>117, 33, 95, 12, 96</td>
<td>W</td>
<td>(P)</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>(O'x)</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>(U, P)</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>N'</td>
</tr>
<tr>
<td>121</td>
<td></td>
<td>N' (U)</td>
</tr>
<tr>
<td>111</td>
<td></td>
<td>b line</td>
</tr>
<tr>
<td>97, 34</td>
<td></td>
<td>Not solved</td>
</tr>
</tbody>
</table>
of various tissues were taken for culture. Table 5 shows the proportions of XY cells in the somatic tissues. The observations on the testes were confined to meiotic cells. The percentages of XY cells in the somatic tissues of Ram 8 which were examined varied: they were low in blood lymphocytes and high in the kidney. In contrast, the percentages in skin and lymphocytes of Ram 47 were similar. The percentages of XY lymphocytes in the live XX/XY rams were approximately constant over the period of observation. In this the lymphocytes differed from the erythrocyte populations, which showed marked changes over 5 years.

There was an indication of a high incidence of non-association (18%) between X and Y chromosomes in first meiotic metaphase of Ram 47, an XX/XY chimaera. Ram 44, the XY/XY chimaera, had only 2% non-association of X and Y chromosomes and none was seen in the normal ram. The numbers of polyploid cells in the testes of the 2 chimaeric and 1 normal ram varied, but it has been suggested by Popescu (1973) that many polyploid spermatocytes in meiotic metaphase may be artefacts of the preparation.

**Sex ratios in offspring**

The two XX/XY chimaeras (Rams 8 and 47) gave rise between them to 18 males and 9 females, whereas the XY/XY chimaera (Ram 44) produced 7 males and 6 females. Ram 46 (XY) also gave rise to twice as many males as females (10:5). The differences from the normal sex ratio were not significant ($\chi^2, P > 0.05$)

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### Table 3. Changes in the red cell populations determined by differential haemolysis in the blood of the chimaeric rams

<table>
<thead>
<tr>
<th>Age</th>
<th>Ram 8 (XX/XY)</th>
<th>Ram 44 (XY/XY)</th>
<th>Ram 47 (XX/XY)</th>
<th>Ram 46 (XY)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blastomere donor (%)</td>
<td>Egg donor (%)</td>
<td>O'x negative</td>
<td>O'x positive*</td>
</tr>
<tr>
<td>2 months</td>
<td>39</td>
<td>53</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>1 year</td>
<td>14</td>
<td>75</td>
<td>36</td>
<td>64</td>
</tr>
<tr>
<td>1½ years</td>
<td>7</td>
<td>94</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>2 years</td>
<td>8</td>
<td>87</td>
<td>23</td>
<td>77</td>
</tr>
<tr>
<td>3 years</td>
<td>7</td>
<td>93</td>
<td>21</td>
<td>79</td>
</tr>
<tr>
<td>4½ years</td>
<td>5</td>
<td>96</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td>5½ years</td>
<td>—</td>
<td>100</td>
<td>1</td>
<td>99</td>
</tr>
</tbody>
</table>

The values in both columns do not add up to exactly 100% because of the unavoidable variability of the method of differential haemolysis. A, N' negative etc. refers to % cells which were negative for that particular reagent.

* Second population inferred: it could not be directly measured because no antibody was available which would lyse the O'x negative population.

### Table 4. Red cell potassium concentrations (mmol/l) in the four rams

<table>
<thead>
<tr>
<th>Age</th>
<th>Ram 8 (XX/XY)</th>
<th>Ram 44 (XY/XY)</th>
<th>Ram 47 (XX/XY)</th>
<th>Ram 46 (XY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 months</td>
<td>67</td>
<td>104</td>
<td>34</td>
<td>105</td>
</tr>
<tr>
<td>1 year</td>
<td>49</td>
<td>104</td>
<td>27</td>
<td>96</td>
</tr>
<tr>
<td>1½ years</td>
<td>35</td>
<td>92</td>
<td>29</td>
<td>90</td>
</tr>
<tr>
<td>2 years</td>
<td>35</td>
<td>92</td>
<td>27</td>
<td>87</td>
</tr>
<tr>
<td>3 years</td>
<td>28</td>
<td>95</td>
<td>27</td>
<td>80</td>
</tr>
</tbody>
</table>
Table 5. The proportions of ‘male’ cells in the four experimental animals and one normal ram

<table>
<thead>
<tr>
<th>Ram</th>
<th>Sex chromosomes</th>
<th>Age (years)</th>
<th>Blood lymphocytes</th>
<th>Skin</th>
<th>Kidney</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of cells examined</td>
<td>% XY</td>
<td>No. of cells examined</td>
<td>% XY</td>
</tr>
<tr>
<td>8</td>
<td>XX/XY</td>
<td>4</td>
<td>50</td>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>50</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>44</td>
<td>XY/XY</td>
<td>4</td>
<td>50</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>100</td>
<td>50†</td>
<td>100</td>
</tr>
<tr>
<td>46</td>
<td>XY</td>
<td>4</td>
<td>50</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>76</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>47</td>
<td>XX/XY</td>
<td>4</td>
<td>50</td>
<td>66</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>100</td>
<td>68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>72</td>
<td>50‡</td>
<td>66</td>
</tr>
<tr>
<td>Normal</td>
<td>XY</td>
<td>6-7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* After 4 days culture.
† After 9 days culture.
‡ After 14 days culture.

Discussion

In studies with tetraparental mice it has been observed that XX/XY chimaeras only transmit male-derived genes to their offspring, presumably because female cells cannot mature in the environment of a male testis (see McLaren, 1976). The present work on tetraparental sheep chimaeras supports these findings, because the 2 rams which were XX/XY chimaeras only passed on genes derived from one cell line (presumably the male line) whereas the ram that was an XY/XY chimaera transmitted genes from both sets of parental lines.

In all 3 ram chimaeras the b-line red cell population declined over the years and virtually disappeared in 2 of the sheep. At the time Ram 8 was mated, he was in fact transmitting a blood type which was not detectable in his own blood. One transferrin line also disappeared in one of the rams. Similar changes have been observed in mouse chimaeras (Mintz & Palm, 1969) and the most likely explanation would be selective advantage of one population over another. On the other hand there has never been any evidence in ‘naturally occurring’ sheep chimaeras due to uterine placental vascular anastomosis that the percentages of the two red cell populations change with age (Dain & Tucker, 1970; E. M. Tucker, unpublished).

In contrast to the findings on the red cell populations, no change was observed in the relative proportions of XX and XY lymphocytes in the 2 XX/XY rams over 5 years. More than one explanation can be put forward to account for this disparity between two cell types (erythrocytes and lymphocytes) which are generally supposed to have originated from a common stem cell. It could be due to the differing selection processes which operate on the two cell types during differentiation or it could merely be the result of the difference in the life spans of the two cell types. The thymus-derived lymphocyte (at least in man and mouse) is a long-lived cell (see White, 1975), and it could therefore take several years before any selection process became apparent; indeed such a selection may account for the very slow change already observed in the proportions of XX and XY lymphocytes in naturally occurring heterosexual twin chimaeras over a similar period of time (Dain, 1974).

References


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