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Genotypically unbalanced diploid ↔ diploid foetal mouse chimaeras: possible relevance to human confined mosaicism

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Summary

Two series of mouse chimaeras were produced by aggregating pairs of eight-cell embryos that differed at the *Gpi-1s* locus, encoding glucose phosphate isomerase (GPI-1); the paired embryos were respectively homozygous *Gpi-1s*^{a}/*Gpi-1s*^{a} and *Gpi-1s*^{b}/*Gpi-1s*^{b}. Chimaeric blastocysts were transferred to pseudopregnant females, that were homozygous *Gpi-1s*^{c}/*Gpi-1s*^{c} and produced only GPI-1C enzyme. Quantitative electrophoresis of GPI-1 was used to estimate the contribution of each embryo (GPI-1A and GPI-1B enzyme activity) to the foetus, placenta and other extraembryonic tissues of 12½ day chimaeric conceptuses. For both series of chimaeras, the distributions of %GPI-1A in different tissues were classified as (1) balanced and typical, (2) balanced but atypical or (3) unbalanced. One series of chimaeras was clearly unbalanced, so that the cells derived from the (C57BL × CBA/Ca)F_2 embryo (*Gpi-1s*^{b}/*Gpi-1s*^{b}) predominated over those derived from the BALB/c inbred strain (*Gpi-1s*^{a}/*Gpi-1s*^{a}) in most foetuses. Two significant observations were made concerning this unbalanced series. Firstly, the mean composition of the placenta and other extraembryonic tissues was similar to that in the foetus, i.e. also unbalanced with (C57BL × CBA/Ca)F_2 (abbreviated to BF_2) cells predominating. Secondly, despite this generalized deficiency of BALB/c cells, there were differences in the frequency of non-chimaeric tissues between different developmental lineages. In 20/34 chimaeric conceptuses in the unbalanced series only BF_2 cells were detected in the foetus, whereas both BF_2 and BALB/c cells were present in at least one of the extraembryonic tissues. This group of chimaeras, therefore, shows some similarities to human confined mosaicism. Although chimaerism occurred more often in the primitive endoderm (hypoblast) lineage (yolk sac endoderm and parietal endoderm) than in the placenta, this may also be the case in human mosaics. The mosaic status of the human yolk sac endoderm is usually unknown so it is possible that mosaicism often occurs in the yolk sac endoderm as well as the trophectoderm in human 'confined placental mosaicism'. The uniformly unbalanced phenotype seen in the mouse chimaeras may be a result of generalized cell selection against BALB/c cells in all tissues. As an alternative explanation, we propose that most of the BALB/c cells in the blastocyst are allocated to the mural trophectoderm, which has a limited mitotic potential and so contributes little to the mid-gestation conceptus. Further work is required to test these possibilities.

1. Introduction

Kalousek & Dill (1983) drew attention to the fact that when chromosome mosaicism occurs in a human conceptus it may not be present in all tissues; in many cases it is confined to the chorionic villus. Subsequent clinical trials, comparing prenatal diagnosis by amniocentesis and chorionic villus sampling (CVS), demonstrated that chromosome mosaicism present in the villus but not in the foetus is a diagnostic problem when prenatal diagnosis is done by chorionic villus sampling. For example, the Canadian collaborative CVS-amniocentesis clinical trial group (1989) showed that the proportion of false positive diagnoses was higher for CVS (2·0%) than for amniocentesis (0·3%). A similar diagnostic problem was highlighted by the MRC working party on the evaluation of chorion villus sampling (1991).

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The abnormal cell population in confined mosaicism is usually trisomic and is more frequently detected in direct chromosome preparations from CVS than in cultured samples. This implies that it more commonly affects the trophoblast than the mesodermal core of the villus (Kalousek, 1990). The trophoblast is derived from the trophectoderm lineage established at the blastocyst stage whereas the mesodermal core of the villus is derived from the inner cell mass of the blastocyst (Luckett, 1978). The reason why the chromosomally abnormal cells are often confined to the chorionic villus is unknown but several possibilities have been discussed by Crane & Cheung (1988). Abnormal cells may arise relatively late in development and either arise or survive preferentially in the trophectoderm lineage. Alternatively, normal and abnormal cells may co-exist in the preimplantation embryo but the abnormal cells may either be preferentially allocated to the trophectoderm in the blastocyst or may be lost subsequently from the other developmental lineages.

Chimaeras differ from mosaics in that the two distinct cell populations are derived from different zygotes (Ford, 1969) but in many other respects they are similar. Mouse chimaeras can be produced by aggregating preimplantation embryos at the 8-cell stage and may provide animal models for some types of human mosaicism, where two genetically distinct cell populations co-exist at an early stage. Although the most relevant models are likely to be those that comprise a mixture of normal diploid cells and chromosomally abnormal cells, other chimaeric combinations may be instructive. For example, Mullen & Whitten (1971) drew attention to the differences between adult chimaeras with 'balanced' and 'unbalanced' genotype combinations. This classification was based on coat pigmentation and unbalanced genotypes were considered to be 'those in which one of the genotypes predominated in most of the animals'. Chimaeras made by aggregating inbred C3HeB/FeJ embryos with (SJL/J × 129/Rr)F_1 embryos formed a genetically unbalanced series, with the (SJL/J × 129/Rr)F_1 component predominating. Similarly, the C57BL/10GnDg component tended to predominate over BALB/cGnDgWt in C57BL/10GnDg ↔ BALB/cGnDgWt chimaeras.

If the deficiency of one cell population in the foetus in an unbalanced strain combination did not occur in the placenta or other extraembryonic tissues these chimaeras might provide a useful model for human confined placental mosaicism. We have studied the composition of foetuses and extraembryonic tissues in a balanced and an unbalanced series of chimaeric mouse conceptuses involving the BALB/c strain. This was done to test whether a predominance of cells from one mouse strain in the foetus was accompanied by a similar predominance in other tissues. The aims of these experiments were to explain the aetiology of unbalanced chimaerism in mice and determine whether it resembled human confined placental mosaicism.

2. Materials and methods

(i) *Production of chimaeras*

Three groups of F_1 mice were produced. One F_1 stock (abbreviated to AF_1) was produced by crossing inbred partly congenic C57BL/Ola.AKR-*Gpi-1s*^{a},*c*/Ws strain females (name abbreviated to BC) with inbred BALB/c males. Another F_1 stock (abbreviated to BF_1) was produced by crossing inbred C57BL/Ws strain females with inbred CBA/Ca males. The AF_1 animals were homozygous for albino (*c*/*c*) and the *Gpi-1s*^{a} allele of the glucose phosphate isomerase locus; the BF_1 animals were homozygous *C*/*C* (pigmented) and *Gpi-1s*^{b}/*Gpi-1s*^{b}. The third F_1 stock (abbreviated to CF_1) was homozygous for albino (*c*/*c*) and the *Gpi-1s*^{c} allele. This was produced by crossing inbred partly congenic C57BL-*Gpi-1s*^{c},*c*/Ws strain females (name abbreviated to CC) with inbred partly congenic BALB/c-*Gpi-1s*^{c}/Ws strain males (name abbreviated to CALB). CBA/Ca males were obtained from the Institute of Cell, Animal and Population Biology, University of Edinburgh; BALB/c and some BF_1 mice were purchased from the Department of Medical Microbiology, University of Edinburgh. All other animals were bred and maintained, under conventional conditions, in the Centre for Reproductive Biology.

BALB/c, AF_1 and BF_1 females were superovulated by injecting 5IU pregnant mares' serum gonadotrophin (PMSG) at approximately 12 noon followed 48 hours later by 5IU human chorionic gonadotrophin (hCG). Females were housed with males from the same inbred or F_1 strain and mating was verified the following morning by the presence of a vaginal plug; the day of the vaginal plug was designated $\frac{1}{2}$ day *post coitum* (*p.c.*). Embryos produced in this way were BALB/c × BALB/c, (AF_1 × AF_1)F_2 and (BF_1 × BF_1) F_2; abbreviated to BALB/c, AF_2 and BF_2 respectively. On the day that the vaginal plugs were found, a group of CF_1 females was examined and those in oestrus were mated to vasectomized CF_1 males to provide homozygous *Gpi-1s*^{c}/*Gpi-1s*^{c} pseudopregnant females.

Preimplantation embryos were flushed from the reproductive tract of pregnant BALB/c, AF_1 and BF_1 females at $2\frac{1}{2}$ days *p.c.* (usually between 9.00 am and 11.00 am) with HEPES-buffered M2 handling medium (Quinn, Barros, & Whittingham, 1982); most were at the 8-cell stage. After removal of the zona pellucida in acidic Tyrodes solution, pH 2·5 (Nicolson, Yanagamachi & Yanagamachi, 1975), each embryo was washed in M2 medium. Aggregation chimaeras were produced by standard procedures involving aggregation of pairs of zona-free 8-cell stage embryos (Tarkowski, 1961; Mintz, 1962; McLaren, 1976).

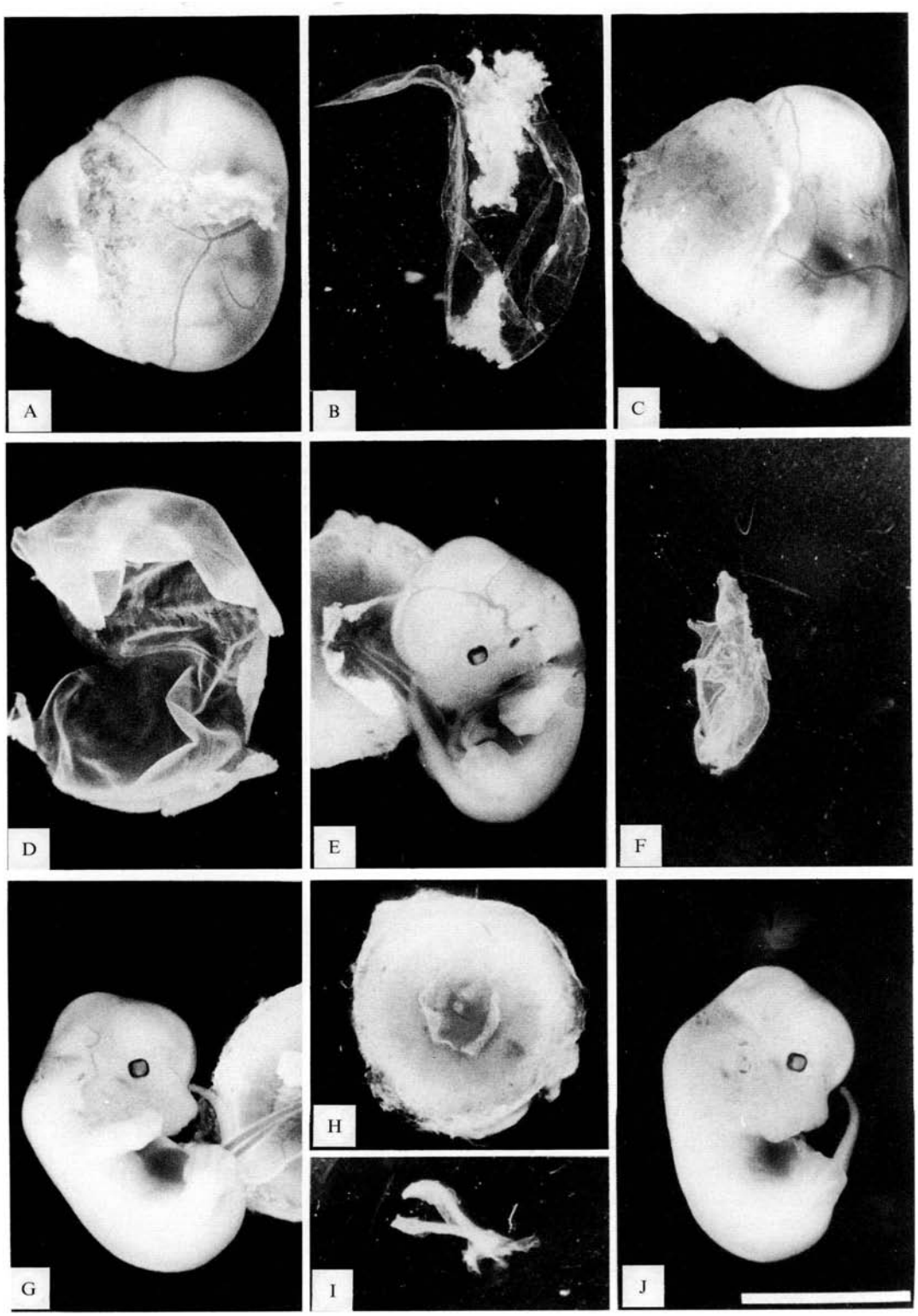

Fig. 1. Photographs showing the various stages in the dissection of a $12\frac{1}{2}$ day conceptus into its component parts. The intact conceptus (A) was removed from the uterus and placed in a drop of M2 handling medium under a dissecting microscope and dissected with fine watchmaker's forceps. First, the outer layer comprising the parietal endoderm cells of Reichert's membrane and a strip of trophoblast and maternal decidua (B) was removed. (The trophoblast and decidual tissue were subsequently separated from Reichert's membrane as shown in Fig. 2.) At this stage, the conceptus (C) was enveloped by the thick visceral yolk sac, which was removed (D) and later separated into mesoderm and endoderm layers as shown in Fig. 3. Next to be removed from the conceptus (E) was the thinner amnion (F) and finally the conceptus (G) was separated into the placenta (H), umbilical cord (I) and fetus (J). The umbilical cord was not used in this study and the remains of any membranes were trimmed from the placenta with fine scissors. Bar = 5 mm.

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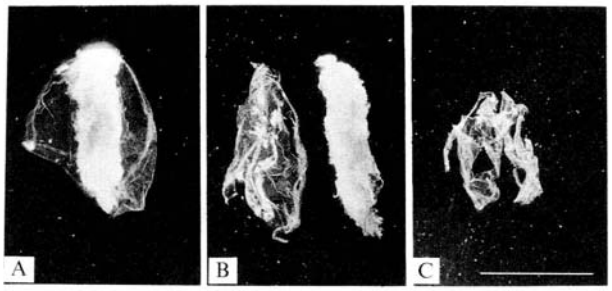

Fig. 2. Illustration of the separation of the outer membrane (A) of the $12\frac{1}{2}$ day mouse conceptus into two parts (B). First, the membrane was placed in a drop of M2 handling medium under a dissecting microscope and most of the strip of trophoblast and maternal decidual tissue (B: right) was peeled off Reichert's membrane (B: left) with fine watchmaker's forceps. Next, the remaining adhering trophoblast and decidual tissue was removed to leave Reichert's membrane (C) with its covering of parietal endoderm cells. Bar = 5 mm.

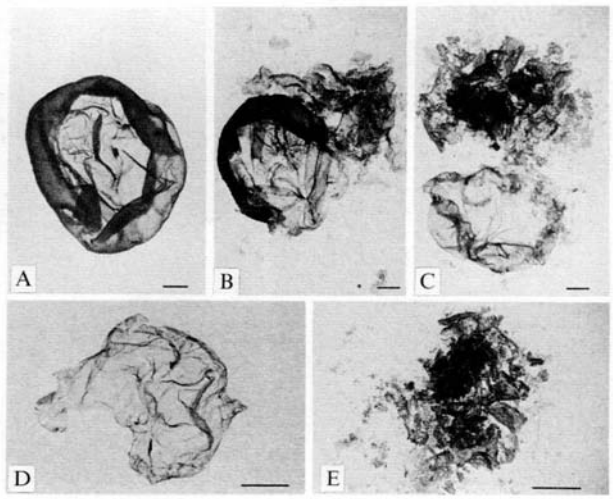

Fig. 3. Illustration of the separation of the yolk sac endoderm and mesoderm layers. The visceral yolk sac was placed in a multiwell plate containing trypsin/pancreatin solution (0·5 g trypsin and 2·5 g pancreatin in 100 ml phosphate buffered saline) and incubated at 4 °C for $2\frac{1}{2}$–3 h (Levak-Svajger, Levak-Svajger & Skreb, 1969). It was then transferred to M2 handling medium and stored at 4 °C (usually for approximately 1 h) until it was dissected. First, the intact yolk sac (A) was placed in a watchglass, containing M2 handling medium, under a dissecting microscope and strips of the darker endoderm layer were peeled off (B) with watchmaker's forceps until the intact mesoderm layer was clean of endoderm (C: endoderm above, mesoderm below). Finally, the mesoderm (D) was rinsed in M2 medium in a clean watchglass, leaving the fragments of endoderm (E) as a separate sample. Bar = 5 mm.

Embryos were transferred in pairs (one AF_2 and one BF_2 embryo in series XM or one BALB/c and one BF_2 embryo in series XR) to drops of culture media under paraffin oil (Boots) in bacteriological grade Petri dishes. Firstly, each pair was pushed together in a drop of M2 + PHA [1 part phytohaemagglutinin (M form, GIBCO 670-0576) plus 19 parts M2 medium] to aid adhesion (Mintz, Gearhart & Guymont, 1973; Pratt, 1987), incubated for 2 minutes at room temperature, then rinsed in a drop of M2. The aggregated embryos were then rinsed in a drop of M16 culture medium (Whittingham, 1971) and transferred to a second drop of M16 medium under paraffin oil, in a dish that had been equilibrated overnight at 37 °C in 5% CO_2 in air. Any pairs of embryos that had drifted apart were pushed together again and the aggregated pairs were cultured overnight. The following day the aggregated embryos were rinsed in M2 handling medium and surgically transferred to the uterus of a CF_1 pseudopregnant female, using a fine glass pipette (McLaren & Michie, 1956). Pseudopregnant females were anaesthetized with 0·25 ml per 30 g body weight of a 1:1 v/v mixture of Hypnorm (0·315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; Janssen Pharmaceuticals) and Hypnovel (2 mg/ml midazlam hydrochloride; Roche). Pregnancies were timed according to the pseudopregnant female.

(ii) *Analysis of chimaeras*

Females were sacrificed at $12\frac{1}{2}$ days gestation and the conceptuses were dissected, as shown in Figs 1–3, to provide foetus, amnion, visceral yolk sac mesoderm, visceral yolk sac endoderm, parietal endoderm (Reichert's membrane), a mixture of trophoblast and maternal decidua (overlying Reichert's membrane) and placenta. The proportions of pigmented and unpigmented cells in the retinal pigment epithelium

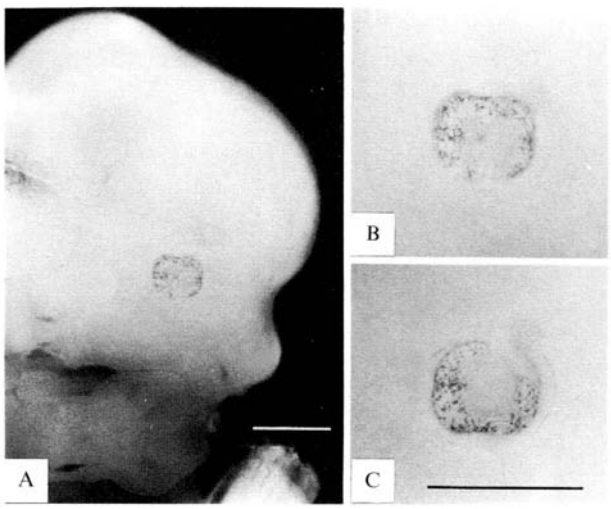

Fig. 4. Variegated pigmentation in the retinal pigment epithelium of the $12\frac{1}{2}$ day fetal eye: (A) head of chimaeric fetus showing eye; (B & C) enlargements of left and right eye showing patches of pigmented and unpigmented cells. Bar = 1 mm.

(Fig. 4) were subjectively estimated unless the foetus was too immature. In addition, weights of the total conceptus, placenta and foetus, crown/rump length and morphological index based on hind limb de-

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velopment (McLaren & Buehr, 1990; Palmer & Burgoyne, 1991) were recorded; these data will be reported elsewhere.

After removal of the foetal heads (for another study), the foetus and placenta were stored at −20 °C in 100 μl of 50% glycerol in water, in 1·5 ml microtubes. All other tissues were stored in 10 μl of 50% glycerol in microtest plates. Samples were lysed by three cycles of freeze/thawing with mechanical disruption of the foetal and placental tissues. Electrophoresis, staining for glucose phosphate isomerase (GPI-1) activity and densitometry (with a Helena Process-24 gel scanner) were carried out as previously described (West, Leask & Green, 1986). The proportions of the two cell populations in the chimaeric tissues were estimated from the proportions of GPI-1A and GPI-1B allozymes. The accuracy of the quantification of the two GPI-1 allozymes by cellulose acetate electrophoresis and scanning densitometry has been validated previously (e.g. West & Green, 1983; James, Flockhart, Keighren & West, 1993). Overall, the estimated %GPI-1A accurately reflects the cell composition in a control mixture or chimaeric tissue, but there is a tendency to overestimate the minor component slightly. GPI-1AB heteropolymer was produced by some chimaeric placentas (to be reported elsewhere); half of %GPI-1AB heteropolymer was added to both %GPI-1A and %GPI-1B values. The raw data (as %GPI-1A allozyme given to one decimal percentage point) was used for statistical analysis and plotting the figures but they were rounded to the nearest integer for presentation in Tables 1 and 2.

(iii) *Statistical analysis*

Non-parametric statistical tests were performed on an Apple Macintosh computer using the statistical packages 'StatView 4.0' (Abacus Concepts Inc., Berkeley, U.S.A.) and 'MultiStat' (Biosoft, Cambridge, U.K.) and a routine established on the spreadsheet Microsoft Excel (Microsoft Corporation).

3. Results

(i) *Balanced and unbalanced strain combinations*

Two series of chimaeras (XM and XR) were produced, each with over 30 chimaeric conceptuses. Quantitative electrophoresis of GPI-1 was used to estimate the contribution of each of the 8-cell stage embryos that were aggregated (GPI-1A and GPI-1B enzyme activity respectively) to the foetus, placenta and other extraembryonic tissues of each $12\frac{1}{2}$ day chimaeric conceptus. Electrophoresis of GPI-1 from chimaeric samples is illustrated in Fig. 5. The use of homozygous *Gpi-1s*^{c}/*Gpi-1s*^{c} recipient females allowed analysis of the chimaeric composition of the various samples without interference from maternal tissue, which was entirely GPI-1C. Maternal GPI-1C was always present in the placentas (Fig. 5a, lanes 1–4) and the samples of trophoblast and decidua that were removed from Reichert's membrane (Fig. 5d, lanes 21–26) and was also present in some of the parietal endoderm samples (e.g. Fig. 5c, lane 15).

The detailed quantitative results are shown in Tables 1 and 2. Data for conceptuses that were non-chimaeric in all tissues assayed are shown in these Tables but were excluded from the graphs and

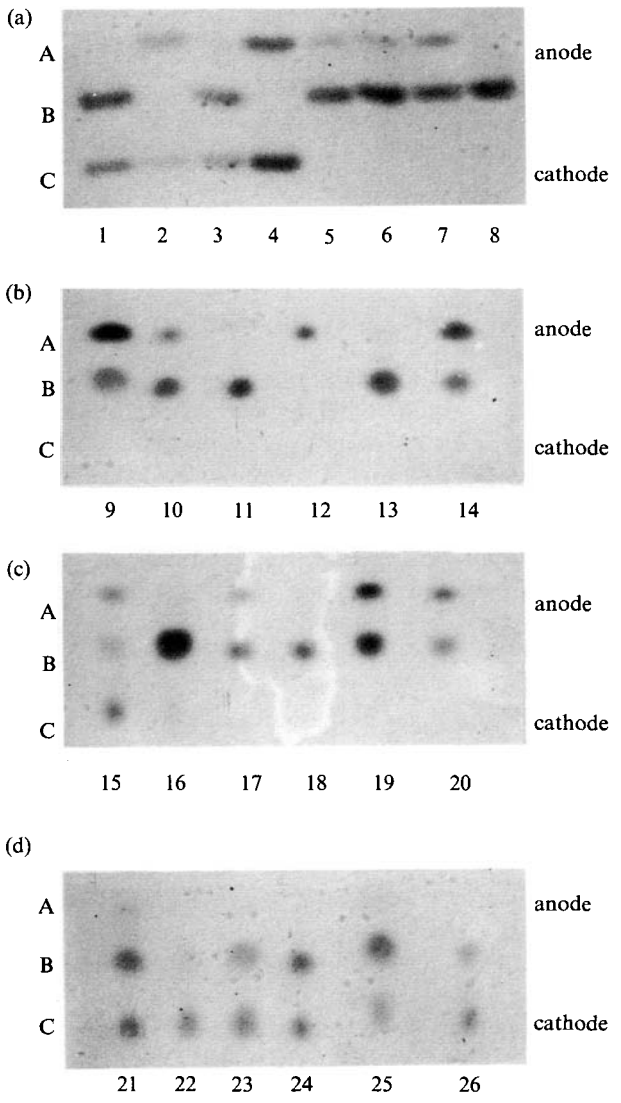

Fig. 5. GPI-1 electrophoresis plates; samples were loaded either with a 0·25 μl applicator (a) or a fine Pasteur pipette (b–d). GPI-1 allozymes (A, B and C) are indicated at the side; migration was towards the cathode. (a) Lanes 1–4, placenta from XR-19, 20, 21 & 22 (maternal GPI-1C present); lanes 5–8, yolk sac endoderm from XR-1, 2, 3 & 4. (b) Lanes 9–14, yolk sac mesoderm from XM-16, 17, 18, 19, 20 & 21 (maternal GPI-1C absent). (c) Lanes 15–20, parietal endoderm from XM-5, 6, 7, 8, 9 and 10 (contaminating maternal GPI-1C present in lane 15). (d) Lanes 21–26, samples of trophoblast plus decidua from XR-32, 33, 34, 35, 36 & 37 (maternal GPI-1C is present in all samples but GPI-1A is only present in lanes 21 and 25; the GPI-1A is barely detectable in the photograph but showed clearly on the original plates and on the scans).

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statistical analysis (below). The dead conceptuses and those with fused placentas were also excluded from the graphs and statistical analysis. Although the fused placentas were separated at dissection, the separation may not have been complete. For some of the conceptuses shown in Tables 1 and 2 the data are incomplete. The percentage pigmentation in the eye was not estimated for immature foetuses and the mixed sample of trophoblast and decidua overlying Reichert's membrane was sometimes exclusively maternal decidua (GPI-1C). Technical losses account for the other missing data.

The mean %GPI-1A (% albino in the eye) in the chimaeric conceptuses (Tables 1 and 2) shows that AF_2 and BF_2 cells are fairly equally represented in all eight tissues studied from the $AF_2 \leftrightarrow BF_2$ chimaeras (series XM). However, in the BALB/c $\leftrightarrow BF_2$ chimaeras (series XR), the BALB/c component is underrepresented in each tissue and typically contributes less than 20%.

The eight tissues analysed are derived from three primary developmental lineages as shown in Fig. 6. The foetus (including the eye), amnion and yolk sac mesoderm are derived from the primitive ectoderm (epiblast) lineage; the yolk sac endoderm and parietal endoderm of Reichert's membrane are derived from the primitive endoderm (hypoblast) lineage and the trophoblast overlying Reichert's membrane is derived from the polar trophectoderm. The placenta is largely a mixture of maternal tissue (which did not affect the analysis because it produced only GPI-1C) and trophoblast, derived from the polar trophectoderm lineage. It has been estimated that placental contributions from other developmental lineages represent only 4% of the non-maternal (zygotic) part of the placenta (Rossant & Croy, 1985). So for the present purposes the placenta can be regarded as essentially a derivative of the polar trophectoderm.

The distributions of the %GPI-1A in a representative tissue (foetus, yolk sac endoderm and placenta) from each of the developmental lineages were plotted as histograms for each of the series of chimaeras (Fig. 7). These histograms indicate that the distributions are reasonably balanced for the $AF_2 \leftrightarrow BF_2$ chimaeras (series XM) but highly skewed in favour of the BF_2 component in the BALB/c $\leftrightarrow BF_2$ chimaeras (XR series). As a population the placentas from series XM were reasonably balanced (mean = 43·0% GPI-1A) but, in individual placentas, either GPI-1A or GPI-1B tended to predominate, giving rise to a bimodal or nearly U-shaped distribution (Fig. 7c).

To demonstrate more objectively that series XR was unbalanced we extended the previously used criteria. Mullen & Whitten (1971) considered that a given strain combination was 'developmentally balanced' if there was a large proportion of animals with a high degree of chimaerism (containing between 30 and 70% of each cell population). The term 'large proportion' was not defined. In our experiments we analysed extraembryonic tissues as well as the foetus, so we revised Mullen & Whitten's definition of a balanced strain combination, which was based entirely on adult coat pigmentation.

In series XM, four conceptuses failed to show chimaerism in any of the tissues analysed; 2 were entirely GPI-1A and 2 were entirely GPI-1B. In series XR, 14 conceptuses failed to show chimaerism in any of the tissues analysed; 2 were entirely GPI-1A and 12 were entirely GPI-1B. Ignoring those conceptuses with fused placentas, the frequency of non-chimaeric conceptuses was not significantly higher in series XR (14/52 versus 4/37; $\chi^2 = 2{\cdot}55$; $P = 0{\cdot}11$) but the 2:12 ratio (GPI-1A:GPI-1B) was significantly different from the 1:1 ratio expected by chance ($\chi^2 = 7{\cdot}14$; $P < 0{\cdot}01$).

Next we considered the chimaeric conceptuses and analysed each tissue separately. We used two criteria to define a strain combination as unbalanced. Classification I was based on that used by Mullen & Whitten (1971). Individuals were divided into three groups according to the %GPI-1A: < 30, 30–70 and > 70%. If the number of individuals in the 30–70% GPI-1A group was not greater than, or equal to, the number in each of the other two groups, the strain combination was considered to be atypical. In classification II, the strain combination was considered to be unbalanced if the number of individuals with < 50% GPI-1A was statistically significantly different from those with > 50% GPI-1A. For this classification, the number of samples with exactly 50·0% GPI-1A (none in this study) would be divided equally between the < 50 and > 50% groups. Using these two criteria, the distributions of %GPI-1A in different tissues were classified as (1) balanced and typical, (2) balanced but atypical or (3) unbalanced. Table 3 shows the tissues from each series of chimaeras classified both ways. With these revised criteria, all of the tissues from series XM were classified as balanced but the trophoblast and placenta were 'atypical' because more samples had < 30% GPI-1A than 30–70% GPI-1A. In contrast, all of the tissues from series XR were classified as unbalanced.

(ii) *Composition of different tissues*

Tables 1 and 2 show good correlations between the % albino in the foetal eye (mean of both eyes) and the %GPI-1A in the rest of the foetus and this is supported by statistical analysis. For series XM, Spearman's rank correlation coefficient, $r_s = 0{\cdot}918$; $P < 0{\cdot}0001$; for series XR, $r_s = 0{\cdot}974$; $P < 0{\cdot}0001$. The %GPI-1A was also very significantly positively correlated between tissues within each of the three primary developmental lineages and also between the primitive ectoderm and trophectoderm lineages in series XR (Table 4). This correlation was not found in series XM

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Table 1. *%GPI-1A (or % albino in the eye) in tissues of 12½ day chimaeric conceptuses in balanced series XM, ranked by %GPI-1A in the foetus*

	Primitive ectoderm lineage*				Primitive endoderm		Trophectoderm	
Chimaera ref.	Eye % albino	Foetus	Amnion	YS Mes	YS End.	P. End.	Troph.	Placenta
XM-30	0	8	12	8	32	29	19	41
XM-27	10	21	12	14	33	44	0	0
XM-6	15	23	22	30	0	0	14	15
XM-17	25	25	38	32	54	67	18	16
XM-37	13	28	22	21	36	48	76	41
XM-34	23	29	28	38	29	39	—	26
XM-2	25	31	20	20	29	77	49	11
XM-7	15	33	31	30	61	27	5	28
XM-36	20	35	44	21	44	27	—	39
XM-25	40	38	18	29	40	59	—	6
XM-33	20	38	37	35	69	46	100	91
XM-22	65	40	28	—	—	40	47	14
XM-3	35	42	43	40	40	20	100	74
XM-41	35	42	42	30	20	22	67	6
XM-4	25	45	51	43	14	48	19	46
XM-32	35	45	50	43	93	100	75	89
XM-9	50	47	56	55	16	41	4	5
XM-15	50	49	43	36	75	47	55	79
XM-38	35	50	33	43	0	0	10	16
XM-16	50	52	62	61	62	75	46	19
XM-39	60	54	31	54	43	91	54	78
XM-5	80	54	59	48	59	59	—	22
XM-24	70	58	50	50	56	86	6	70
XM-1	55	58	52	58	52	32	100	80
XM-13	80	58	64	58	22	55	0	7
XM-11	70	65	70	66	9	18	44	73
XM-31	75	69	54	55	45	66	100	95
XM-40	70	76	79	64	36	0	87	77
XM-35	90	76	71	67	64	62	—	16
XM-29	55	80	63	69	60	33	97	95
XM-8	95	82	57	86	0	0	0	85
XM-28	97	84	68	66	48	83	0	5
XM-10	100	100	100	100	59	46	61	54
Mean	47·9	49·5	45·6	45·9	40·6	45·1	44·7	43·0
S.E.	4·9	3·7	3·6	3·7	4·1	4·7	7·0	5·7
S.D.	28·3	21·0	20·6	20·7	23·0	26·9	36·8	32·8
N	33	33	33	32	32	33	28	33
Coeff. var.	58·9	42·4	45·1	45·1	56·6	59·8	82·3	76·3
Non-chimaeric conceptuses								
XM-18	0	0	0	0	0	0	0	0
XM-26	0	0	0	0	0	—	—	0
XM-19	100	100	100	100	100	100	100	100
XM-23	100	100	100	—	—	100	100	100
Conceptuses with fused placentas								
XM-12A	15	16	22	19	14	49	38	6
XM-12B	—	31	36	25	0	—	—	0
XM-20	3	6	6	4	13	12	4	4
XM-21	65	74	64	57	42	70	87	72
Dead conceptus								
XM-14	—	26	38	34	20	50	—	80

* The tissues are arranged according to their developmental origin from the primitive ectoderm (epiblast), primitive endoderm (hypoblast) or trophectoderm lineage. Abbreviations: YS Mes, yolk sac mesoderm; YS End., yolk sac endoderm; P. End., parietal endoderm; Troph., trophoblast overlying Reichert's membrane.

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Table 2. *%GPI-1A (or % albino in the eye) in tissues of 12½ day chimaeric conceptuses in unbalanced series XR, ranked by %GPI-1A in the foetus*

	Primitive ectoderm lineage*				Primitive endoderm		Trophectoderm	
Chimaera ref.	Eye % albino	Foetus	Amnion	YS Mes	YS End.	P. End.	Troph.	Placenta
XR-8	0	0	0	0	0	24	—	0
XR-37	0	0	0	0	3	5	0	0
XR-40	0	0	0	0	8	0	0	0
XR-16	0	0	0	0	9	10	0	0
XR-12	0	0	0	0	11	10	0	0
XR-25	0	0	0	0	14	7	0	0
XR-43	0	0	0	0	14	0	0	0
XR-33	0	0	0	0	15	31	0	0
XR-51	0	0	0	0	18	28	0	0
XR-2	0	0	0	0	20	32	0	0
XR-35	0	0	0	0	22	45	0	0
XR-17	—	0	0	0	25	42	0	0
XR-54	0	0	0	0	31	38	0	0
XR-11	0	0	0	0	20	0	2	0
XR-7	0	0	0	0	19	9	5	0
XR-1	0	0	0	0	25	15	8	0
XR-36	—	0	0	0	25	41	9	0
XR-45	0	0	0	0	2	0	0	4
XR-38	0	0	0	0	0	10	51	67
XR-18	—	0	9	0	0	9	0	0
XR-10	1	3	0	0	9	19	56	39
XR-9	8	7	11	8	0	0	18	68
XR-14	—	8	15	5	42	14	0	0
XR-46	8	10	14	17	3	4	0	0
XR-19	20	12	7	24	38	38	0	5
XR-6	20	12	18	30	37	70	74	75
XR-4	3	13	0	5	0	25	0	0
XR-32	20	17	5	20	0	0	15	7
XR-52	30	24	22	23	27	31	0	0
XR-23	30	25	23	24	14	42	29	86
XR-41	20	26	31	34	13	54	37	17
XR-44	20	35	34	33	5	7	0	0
XR-34	30	43	17	23	35	35	0	0
XR-21	15	50	28	36	9	42	10	13
XR-27	60	59	51	52	0	0	0	23
XR-39	80	69	85	83	9	42	90	66
XR-20	75	76	72	59	53	34	100	100
XR-3	100	88	78	70	33	26	100	100
Mean	15·8	15·1	13·7	14·3	16·0	22·0	16·3	17·6
S.E.	4·4	3·9	3·7	3·5	2·2	3·0	4·9	5·1
S.D.	25·9	24·0	22·8	21·7	13·7	18·2	30·1	31·7
N	34	38	38	38	38	38	37	38
Coeff. var.	163·6	158·8	166·7	152·1	85·9	82·9	184·0	180·1
Non-chimaeric conceptuses								
XR-5	0	0	0	0	0	0	0	0
XR-13	0	0	0	0	0	0	0	0
XR-15	0?	0	0	0	0	0	0	0
XR-24	0	0	0	0	0	0	0	0
XR-28	0	0	0	0	0	0	0	0
XR-42	0	0	0	0	0	0	0	0
XR-47	0	0	0	0	0	0	0	0
XR-50	0	0	0	0	0	0	0	0
XR-53	0	0	0	0	0	0	0	0
XR-55	0	0	0	0	0	0	0	0
XR-56	0	0	0	0	0	0	—	0
XR-57	0	0	0	0	0	0	0	0
XR-22	100?	100	100	100	100	100	100	100
XR-26	100	100	100	100	100	100	100	100

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Table 2 (*cont.*)

Chimaera ref.	Primitive ectoderm lineage*				Primitive endoderm		Trophectoderm	
	Eye % albino	Foetus	Amnion	YS Mes	YS End.	P. End.	Troph.	Placenta
Conceptuses with fused placentas								
XR-29	1	7	7	13	10	30	18	0
XR-30	0	0	0	11	12	30	64	59
XR-31†	0	0	0	0	0	0	18	39
XR-48	0	0	0	0	0	0	0	0
XR-49	0	0	0	0	0	0	0	0

* The tissues are arranged according to their developmental origin from the primitive ectoderm (epiblast), primitive endoderm (hypoblast) or trophectoderm lineage. Abbreviations: YS Mes, yolk sac mesoderm; YS End., yolk sac endoderm; P. End., parietal endoderm; Troph., trophoblast overlying Reichert's membrane.
† The placenta of conceptus XR-31 was fused to a resorbing mole.

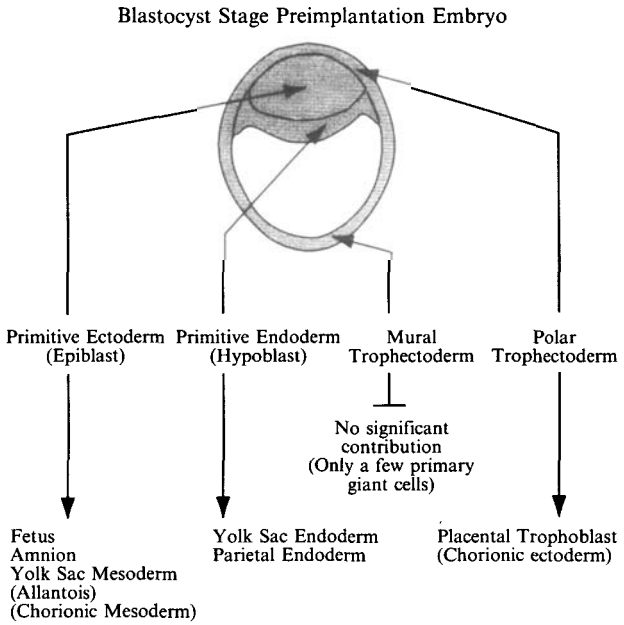

Fig. 6. Diagram showing the derivation of the tissues analysed from three primary developmental lineages at the blastocyst stage (see Gardner & Papaioannou, 1975). The trophectoderm of the blastocyst surrounds the inner cell mass which produces the primitive ectoderm (epiblast) and primitive endoderm (hypoblast) developmental lineages. The mural trophectoderm (adjacent to the blastocoele cavity) makes no significant cellular contribution to the mid-gestation conceptus but the polar trophectoderm (overlying the inner cell mass) produces the placental trophoblast and the chorionic ectoderm. The samples analysed from the primitive ectoderm lineage were the fetus (and eye), amnion and yolk sac mesoderm. The primitive endoderm samples were the yolk sac endoderm and the parietal endoderm layer of Reichert's membrane. The trophoblast (mixed with maternal decidua) overlying Reichert's membrane was derived from the polar trophectoderm lineage as was most of the non-maternal (zygotic) part of the placenta. According to Rossant & Croy (1985), 96% of the zygotic part of the placenta is derived from the polar trophectoderm and the remaining 4% is derived from the inner cell mass. The origin of the minor placental components (allantois, chorionic mesoderm and chorionic ectoderm) is shown in parentheses.

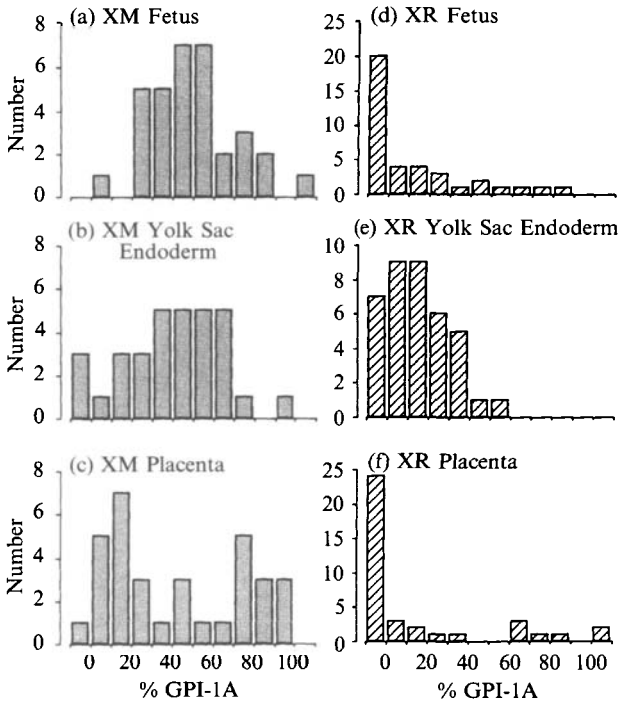

Fig. 7. Distributions of %GPI-1A in three representative tissues (one from each of the three primary developmental lineages) analysed in each series of chimaeric conceptuses (XM and XR). Tissues with either 0 or 100% GPI-1A are shown separately at either end of the distributions.

but there was a weak positive correlation between the yolk sac endoderm and the two trophectoderm-derived tissues.

(iii) '*Confined mosaicism*' *in the unbalanced series of chimaeras*

The results presented above demonstrate that, in series XR, each tissue was unbalanced with BF_2 cells predominating over BALB/c. However, consideration of the data for individual chimaeras (Tables 1 and 2) indicates that there are some differences between

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Table 3. *Chimaeric conceptuses grouped according to the %GPI-1A in each tissue and classified in two ways*

Tissue	Classification I (%GPI-1A)			Classification II (%GPI-1A)		Statistical significance†
	< 30	30–70	> 70	< 50	> 50	χ^2 value
Balanced distributions						
XM Foetus	6	21	6	18	15	0·27
XM Amnion	8	22	3	18	15	0·27
XM Yolk sac mesoderm	7	23	2	18	14	0·50
XM Yolk sac endoderm	10	20	2	20	12	2·00
XM Parietal endoderm	10	17	6	21	12	2·45
Balanced but atypical distributions‡						
XM Trophoblast	12	8	8	16	12	0·57
XM Placenta	16	6	11	20	13	1·48
Unbalanced distributions						
XR Foetus	31	5	2	34	4	23·68*
XR Amnion	32	3	3	34	4	23·68*
XR Yolk sac mesoderm	31	6	1	34	4	23·68*
XR Yolk sac endoderm	31	7	0	37	1	34·11*
XR Parietal endoderm	23	15	0	36	2	30·42*
XR Trophoblast	30	3	4	31	6	16·89*
XR Placenta	30	4	4	31	7	15·16*

* $P < 0{\cdot}001$; others not significantly different.
† Tested against the expectation of equal proportions of samples with < 50% GPI-1A and > 50% GPI-1A in classification II.
‡ Classified as atypical because, in classification I, there were fewer individuals with 30–70% GPI-1A than in one of the other categories.

tissues in the extent of chimaerism, despite the similarities in the mean values. This is also illustrated by the distributions shown in Fig. 7. For example, in the unbalanced series XR, two cell populations are more often present in the yolk sac endoderm (31 of 38 chimaeric conceptuses) than in the fetus (18/38) or placenta (12/38). Although there were fewer yolk sac endoderm samples with 0% GPI-1A, there were also fewer with > 50% GPI-1A. Pooling samples within a lineage, mixed cell populations occurred more frequently in the primitive endoderm derivatives (31/38) than the primitive ectoderm (19/38) or trophectoderm (16/38) tissues.

Table 5 shows that confined mosaicism (strictly speaking, 'confined chimaerism') occurs more frequently in series XR than XM but that chimaerism is either confined to the primitive endoderm plus trophectoderm lineages (group e in Table 5) or confined to just the primitive endoderm lineage (group f) rather than confined to the placenta or trophectoderm lineage alone.

4. Discussion

(i) *Balanced and unbalanced strain combinations*

In the present study two criteria were applied to chimaeric conceptuses, to classify the distributions of %GPI-1A in different tissues as (1) balanced and typical, (2) balanced but atypical or (3) unbalanced. All tissues in series XR were classified as significantly unbalanced. All tissues from XM series conceptuses were classified as balanced but the distributions for the trophoblast and placenta samples were atypical. These balanced but atypical distributions (bimodal or U-shaped) for the two trophoblast derivatives were similar to that reported for the placentas from another series of chimaeras (series XE in James *et al.* 1993) and may be characteristic of these tissues.

Series XR also differed from XM in several other respects and further studies should reveal whether any of these are typical of unbalanced strain combinations. For example, unbalanced strain combinations could be characterized by (1) a high proportion of chimaeric conceptuses with non-chimaeric primitive ectoderm lineages, (2) a significant positive correlation between the compositions of the primitive ectoderm and trophectoderm lineages in chimaeric conceptuses and (3) a significant difference in the frequencies of the two classes of non-chimaeric conceptuses.

(ii) *Origin of the unbalanced chimaeric phenotype*

One aim of these experiments was to explain the aetiology of unbalanced chimaerism in mice. If, in the unbalanced series XR, the unbalanced composition arose entirely as a result of a generalized cell selection against the BALB/c strain genotype, then BALB/c cells should be deficient in all of the extraembryonic tissues as well as the foetus. If, however, the deficiency

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Table 4. *Spearman rank correlation coefficients (r_s), with P values below, for %GPI-1A in different tissues of chimaeric conceptuses. Tissues are grouped according to their origin from primitive ectoderm, primitive endoderm or trophectoderm developmental lineages*

	Primitive ectoderm		Primitive endoderm		Trophectoderm	
	Amnion	Yolk sac mesoderm	Yolk sac endoderm	Parietal endoderm	Trophoblast	Placenta
Series XM						
Primitive ectoderm lineage						
Foetus	0·880	0·934	0·190	0·089	0·067	0·345
	P < 0·0001	*P < 0·0001*	P = 0·2902	P = 0·6140	P = 0·7290	P = 0·0507
Amnion	—	0·885	0·220	0·039	0·047	0·258
		P < 0·0001	P = 0.2213	P = 0·8270	P = 0·8085	P = 0·1445
Yolk sac mesoderm	—	—	0·137	0·038	0·011	0·324
			P = 0·4468	P = 0·8345	P = 0·9545	P = 0·0709
Primitive endoderm lineage						
Yolk sac endoderm	—	—	—	0·517	0·411	0·353
				P = 0·0040	*P = 0·0359*	*P = 0·0491*
Parietal endoderm	—	—	—	—	0·0003	−0·061
					P = 0·9989	P = 0·7311
Trophectoderm lineage						
Trophoblast (over Reichert's membrane)	—	—	—	—	—	0·643
						P = 0·0008
Series XR						
Primitive ectoderm lineage						
Foetus	0·914	0·970	0·089	0·263	0·441	0·625
	P < 0·001	*P < 0·0001*	P = 0·5875	P = 0·1103	*P = 0·0081*	*P = 0·0001*
Amnion	—	0·948	0·110	0·244	0·411	0·596
		P < 0·0001	P = 0·5052	P = 0·1379	*P = 0·0136*	*P = 0·0003*
Yolk sac mesoderm	—	—	0·120	0·289	0·437	0·640
			P = 0·4649	P = 0·0792	*P = 0·0088*	*P < 0·0001*
Primitive endoderm lineage						
Yolk sac endoderm	—	—	—	0·557	0·074	−0·049
				P = 0·0007	P = 0·6589	P = 0·7657
Parietal endoderm	—	—	—	—	0·278	0·196
					P = 0·0949	P = 0·2324
Trophectoderm lineage						
Trophoblast (over Reichert's membrane)	—	—	—	—	—	0·792
						P < 0·0001

The %GPI-1A was considered to be significantly correlated between two tissues when $P < 0·05$ (shown in italics)

in the foetus was a result of a preferential allocation of BALB/c cells to other developmental lineages, more variability would be expected between tissues. For example, at the blastocyst stage, BALB/c cells might be less frequently allocated to the primitive ectoderm (epiblast) developmental lineage than to either the primitive endoderm (hypoblast) or trophectoderm lineages. If so, BALB/c cells would tend to be deficient in all the derivatives of the primitive ectoderm (including the foetus, amnion and yolk sac mesoderm) but they might be expected to predominate in the derivatives of the primitive endoderm (e.g. yolk sac endoderm and parietal endoderm) and trophectoderm (e.g. placental trophoblast).

The results for series XR showed that there were many more cases where BALB/c cells were excluded from all three primitive ectoderm tissues than from only one of them. This suggests that BALB/c cells may be often excluded from the entire primitive ectoderm lineage at the blastocyst stage. However, this was not reflected by the predicted reciprocal predominance of BALB/c cells in the other two lineages. Although there were more cases where BALB/c cells were completely excluded from the primitive ectoderm than the primitive endoderm derivatives (compare groups e and f with group c in Table 5), the mean BALB/c contribution was only slightly higher in the two primitive endoderm tissues (Table 2).

These two key observations do not precisely fit either of the simple models outlined above. The uniformly low mean contribution of BALB/c cells to chimaeric conceptuses suggests that there could be a generalized selection against BALB/c cells throughout the conceptus. The high frequency of conceptuses with chimaerism confined to either the primitive

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Table 5. *Frequencies of chimaerism (mixed cell populations) in the three primary developmental lineages of 12½ day mouse chimaeras (data from Tables 1 and 2, excluding conceptuses with fused placentas)*

GPI-1 composition (mixed or single)			Number of conceptuses	
Primitive ectoderm (epiblast) lineage*	Primitive endoderm (hypoblast) lineage*	Trophecto-derm lineage*	Series XM (balanced) No. (%)	Series XR (unbalanced) No. (%)
Chimaeric foetuses				
(a) Mixed	Mixed	Mixed	28 (76)	7 (13)
(b) Mixed	Mixed	Single	1 (3)	8 (15)
(c) Mixed	Single	Mixed	3 (8)	3 (6)
Non-chimaeric foetuses (chimaerism confined to extraembryonic tissues)				
(d) Mixed	Mixed	Single	0	1† (2)
(e) Single	Mixed	Mixed	1 (3)	6 (12)
(f) Single	Mixed	Single	0	13 (25)
Non-chimaeric conceptuses				
(g) Single	Single	Single	4 (11)	14 (27)
Total number of chimaeric conceptuses			33	38
Total number of conceptuses			37	52

* Primitive ectoderm (epiblast) lineage samples: foetus, amnion and yolk sac mesoderm. Primitive endoderm (hypoblast) lineage samples: parietal endoderm of Reichert's membrane and yolk sac endoderm. Both the primitive ectoderm and primitive endoderm lineages are derived from the inner cell mass of the blastocyst. Trophectoderm lineage samples: trophoblast overlying Reichert's membrane and placenta.

† Chimaera XR-18 had both GPI-1A and GPI-1B activity in the amnion and parietal endoderm but only GPI-1B activity (BF_2 cells) in the foetus and other tissues analysed.

endoderm or primitive endoderm plus trophectoderm suggests two possibilities. BALB/c cells may be at an even greater disadvantage in the foetus, amnion and yolk sac mesoderm and so often become reduced below the limits of detection. Alternatively, BALB/c cells may be preferentially allocated to the primitive endoderm or trophectoderm lineage in the blastocyst. The low mean contribution of BALB/c cells to the primitive endoderm derivatives in series XR (Table 2) makes it unlikely that BALB/c cells are preferentially allocated to this lineage. However, the same need not be true for the trophectoderm as explained below.

The trophoblast cells present at 12½ days are almost all derived from the polar trophectoderm of the blastocyst (Fig. 6) because the mural trophectoderm cells in the blastocyst stop dividing soon after losing contact within the inner cell mass (Copp, 1978, 1979). According to Handyside (1978) and Cruz & Pederson (1985), some of the polar trophectoderm cells are, in turn, derived from the underlying inner cell mass cells. (This could account for the positive correlation between the composition of the primitive ectoderm and trophectoderm derivatives seen in series XR.)

The following explanation may account for the low contribution to all three developmental lineages in series XR. If most BALB/c cells were allocated to the trophectoderm, leaving relatively few BALB/c cells in the inner cell mass (ICM) to contribute to the primitive ectoderm and primitive endoderm lineages, this would explain the low contribution to these two lineages. A low contribution of BALB/c cells to the ICM could frequently result in their complete exclusion from the primitive ectoderm if, for example, more ICM cells were allocated to the primitive endoderm than the primitive ectoderm or if the few BALB/c cells, within the ICM, were preferentially allocated to the primitive endoderm. Even if the trophectoderm was predominantly BALB/c, this may not be reflected by a predominance of BALB/c cells in the 12½ day placenta and trophoblast if most of the BALB/c cells were in the mural trophectoderm (Fig. 6). BALB/c trophectoderm cells would be displaced from the polar trophectoderm to the mural trophectoderm region if cells from the ICM (predominantly BF_2 cells) moved outwards and became polar trophectoderm cells. The polar trophectoderm may then be predominantly composed of BF_2 cells, like the primitive ectoderm and primitive endoderm. Further studies are needed to test this possibility.

(iii) *Similarities between unbalanced chimaeras and human confined mosaicism*

Despite the uniformly low mean contribution of BALB/c cells to all the tissues of the chimaeric conceptuses, our series of unbalanced diploid↔

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diploid mouse chimaeras may provide a useful model for human confined mosaicism. There are similarities between the human condition and those individual mouse conceptuses from the unbalanced series, in which chimaerism was confined to the primitive endoderm and/or trophectoderm lineage, and these similarities may be instructive. Since the mosaic status of the human extraembryonic endoderm is usually unknown, it is possible that mosaicism often occurs both in the trophectoderm and yolk sac endoderm in human 'confined placental mosaicism'. In six chimaeras (12% of the total) both trophectoderm and primitive endoderm lineages were chimaeric in the absence of foetal chimaerism (Table 5, line e) and these chimaeras could parallel human conceptuses classified as confined placental mosaics. It would, therefore, be worth investigating whether chromosomally abnormal cells that appear to be confined to the human placenta are also present in the yolk sac endoderm. This may indicate whether some cases of human confined placental mosaicism result from exclusion of chromosomally abnormal cells from the primitive ectoderm lineage at an early stage.

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