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Original Article

Cell mixing during the early development of mouse aggregation chimera

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ABSTRACT Two different inbred strain combinations of mouse aggregation chimeras – C3H/HeN (H-2^k) x C57BL/6N (H-2^b) and C3H/HeN x BALB/cA (H-2^d) were used for cell mixing analysis at two points in time – 24 h after aggregation (just prior to transplantation into foster mothers) and 7.5 days post coitum (p.c.). The cell proportion of two H-2 haplotypes at the blastocyst stage was studied using a fluorescence-labeled monoclonal antibody recognizing a C3H-specific alloantigen – CSA (C3H strain-specific antigen) and laser scanning confocal microscopy. The 7.5-day-old chimeras were sectioned and subsequently processed by sensitive biotinylated antibody – avidin peroxidase immunohistochemical technique. Our results showed that 24 h after aggregation (blastocyst stage), there was equal cell mixing and no mouse strain used in the present study was dominant at this time. In 7.5-day-old C3H/HeN x BALB/cA chimeras, cells of both genotypes were intermingled, but the C3H/HeN strain was dominant in all cases. In contrast, the combination C3H/HeN x C57BL/6N clearly showed reduced numbers of C3H/HeN cells (CSA-positive) in 83% of the chimeras evaluated. Generally, CSA positive cells were found only in randomly distributed small distinct areas representing less than 20% of embryonal cells. Surprisingly, the extraembryonal ectoplacental cone was uniformly CSA positive in some C3H/HeN x C57BL/6N chimeras. Furthermore, in 36% of normally implanted chimeras of both strain combinations progressive degeneration was observed. We suggest that the cell mixing pattern as well as the absolute number of cells derived from each strain in the aggregation chimera can be affected by specific immune interactions involving H-2 haplotype combinations of the allogeneic fetus and the fully immunocompetent host organism, at later points in development.

KEY WORDS: *mouse chimera, aggregation, H-2 haplotype, cell mixing*

Introduction

Mouse aggregation chimeras continue to be exciting models for studies of early mammalian development. Experimental chimeras have been used to analyse coat-color patterns (Tachi *et al.*, 1990), developmental ability of parthenogenetic stem cells (Jägerbauer *et al.*, 1992), embryonic stem cell fate (Nagy *et al.*, 1990), morphogenesis of neural tissue (Crandall and Herrup, 1990; Crusio *et al.*, 1990) and skeletal muscle differentiation (Fundele *et al.*, 1994), cell-cell contact and interactions (Sepulveda and Izquierdo, 1990; Matta, 1991), and lethal mutations (Barsh *et al.*, 1990). Moreover, in combination with useful cell lineage markers, it is an excellent experimental model for cell differentiation and fate analysis (Deltour *et al.*, 1991; Musci and Mullen, 1992) or studies of the role of cell membrane molecules during early development (Barnes, 1976; Matsunaga *et al.*, 1980).

However, there are few data about genotype interaction and cell mixing in the different strain combinations of mouse aggregation chimeras. The relative lack of reproducible results has been caused by the lack of an optimal histological marker up to the present. Kusakabe *et al.* (1988) and Yoshiki *et al.* (1993) have proposed an almost ideal mouse strain-specific marker: CSA. By using a monoclonal antibody reacting with this antigen, Yoshiki *et al.* (1993) confirmed that cell mixing in aggregation chimeras occurs very early in embryogenesis. In the combination C3H/HeN x BALB/cA, a well intermingled pattern was observed in all embryonic tissues including blood islands at day 7.5 p.c.

A critical point during pregnancy is the implantation of the blastocyst when the embryo attaches to the wall of the uterus

Abbreviations used in this paper: CSA, C3H strain-specific antigen; NGS, normal goat sera; MHC, major histocompatibility complex; p.c., post coitum.

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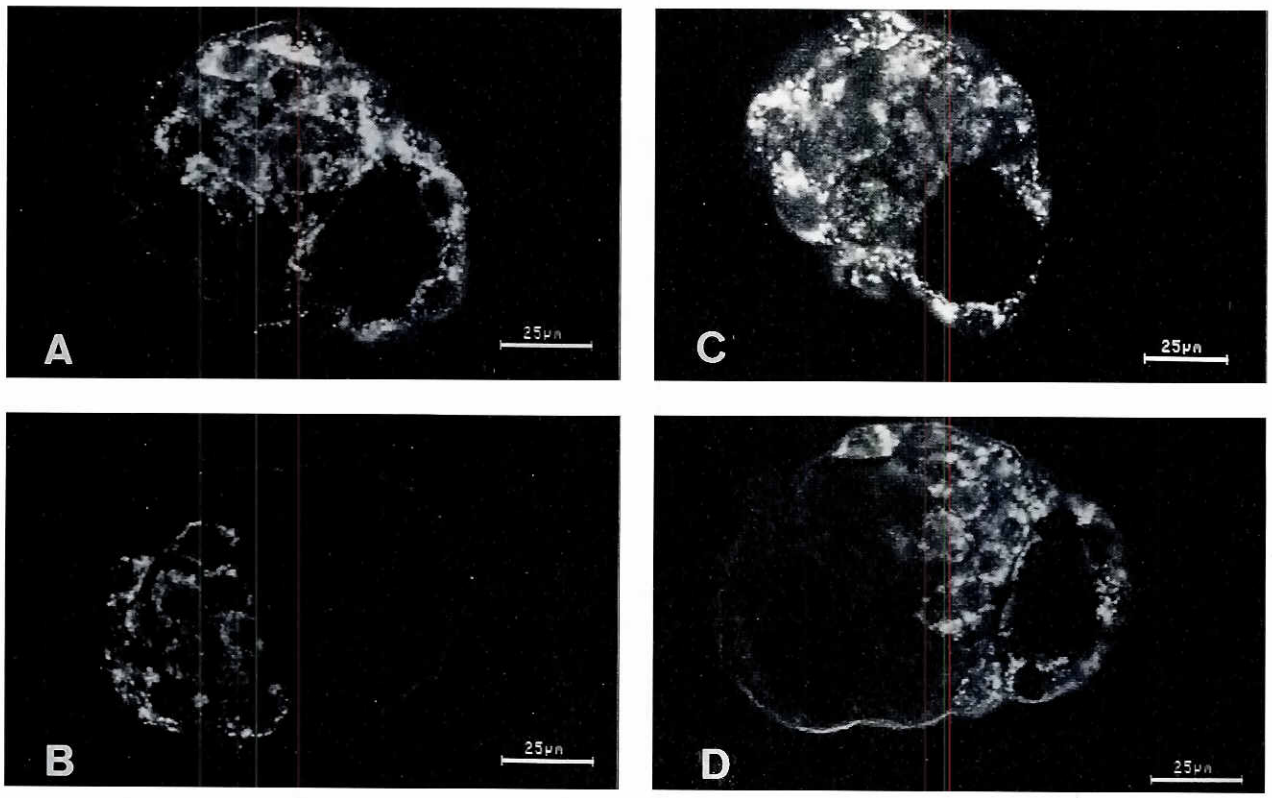

Fig. 1. Cell mixing in preimplantation chimeras. (A,B,C,D). *Immunofluorescence staining of well developed C3H/HeN x C57BL/6N* **(A)** *and C3H/HeN x BALB/cA* **(B)** *chimeric blastocysts 24 h after aggregation. The stained cells are of the C3H genotype while negative cells are of the C57BL or BALB/c genotype. It is important to note that only one representative optical section is shown. When the blastocysts were optically sectioned and genotype ratios at each optical cut were determined, the total cell ratio was equal. Uniformly distributed C3H-specific cell marker – CSA in the control (C3H/HeN x C3H/HeN) aggregates* **(C)** *confirmed specificity of the system. Immunofluorescence staining and morphology of incorrectly developed chimeric blastocyst; in this case with 2 blastocoels* **(D)**. *Such aggregates were excluded from these experiments.*

and becomes directly dependent on the maternal environment. Little is known about the immune regulation of implantation and subsequent specific maternal-fetal immune interactions, thus the making of aggregation chimeras from embryos of defined H-2 haplotypes followed by transplantation into foster mothers, seems to be a useful strategy.

It was originally supposed that embryonal cells might escape recognition by the mother's immune system by total suppression of the expression of transplantation antigens (Billingham, 1964). However in the mouse, MHC class I genes are transcribed in the early embryo (David-Watine *et al.*, 1987; Silverman *et al.*, 1988; Hedley *et al.*, 1989; Drezen *et al.*, 1992, 1994) as well as in the yolk-sac and placental tissues (Philpott *et al.*, 1988). Potential alloantigens are expressed in extraembryonic tissues intimately associated with maternal tissues and blood supply and yet fail to induce immunological rejection (Philpott *et al.*, 1988). Thus, regulated expression of MHC antigens by the placental trophoblast cells, which intervene between the embryo and maternal blood and tissues, is now believed to play an important role in the phenomenon of maternal-fetal immune adaptation (Kydd *et al.*, 1991; Hunt and Orr, 1992). Similarly, in the human the expression of leukocyte antigen A, B, and C loci is greatly reduced in trophoblast cells compared to embryonic cells. When these cells are cocultured with peripheral blood lymphocytes from corresponding females in the presence of IL-2, allogeneic cytotoxic T lymphocytes are generated only in culture with embryonic cells, and trophoblast cells express resistance to lysis (Yahata *et al.*, 1990).

However, there are strong differences between maternal-fetal interactions after natural mating and host-fetal interactions in artificial embryo transfer experiments. As shown in rat allogeneic natural matings, the allele-specific class I transplantation antigens are not expressed on the membrane of the trophoblast but they are expressed after embryo transfer involving embryos of the same genotype (Kanbour-Shakir *et al.*, 1990). These exper-

TABLE 1

RESULTS OF AGGREGATION EXPERIMENTS*

Mouse strain combination	Total number of aggregates		No. of chimeras developed well to the blastocyst stage		No. of blastocysts fixed and stained	
	microholes	PHA-P	microholes	PHA-P	microholes	PHA-P**
C3H/HeN x C57BL/6N	102	26	94	22	12	22
C3H/HeN x BALB/cA	123	26	103	23	13	23

*All aggregates were microscopically examined after 24 h of cultivation in M 16. **Aggregation techniques using PHA-P or microholes were used with similar results.

TABLE 2

RESULTS OF TRANSPLANTATION EXPERIMENTS*

Mouse strain combination	Transferred into		Decidual reaction		Rejected after implantation**		No. of embryos developed well***	
	CD-1	F1	CD-1	F1	CD-1	F1	CD-1	F1
C3H/HeN x C57BL/6N	62	20	32	5	13	1	19	4
C3H/HeN x BALB/cA	70	20	31	2	11	-	20	2

*To exclude the possibility that aggregation technique using PHA-P can stimulate the expression H-2 antigens, only blastocysts aggregated in microholes were transferred into CD-1 or F1 pseudopregnant females. **Decidual reaction was observed, but no embryo was found.***Morphologically normal embryos were sectioned through and 5-10 of representative slides were used for immunostaining.

iments suggest that in the rat the suppression of allele-specific MHC class I antigens on trophoblast cells occurs shortly after fertilization and that it probably requires the uterine environment of natural mating.

In the light of these results it seems possible that the absolute number of cells coming from aggregation partners and final cell mixing can be influenced by the stimulated pseudo-mother's immune system, depending on the H-2 haplotype of both partners.

We report here an analysis of cell mixing using a mouse strain-specific marker in the chimera 24 h after aggregation and 7.5 days p.c. Our report focuses on differences between two inbred strain combinations of aggregation chimera. An additional aim of this study was to determine whether significant changes or tendencies in the ratio and absolute number of cells occurs 5 days after transplantation into females made pseudopregnant by mechanical stimulation.

Results

70 well developed C3H/HeN x C57BL/6N or BALB/cA chimeras were used to analyze the cell mixing pattern 24 h after aggregation – at the blastocyst stage. As revealed by C3H/HeN-specific immunochemical staining and confocal microscopy, no selection against any H-2 haplotype was observed. Similar contribution of both parental genotypes in both strain combinations was found in the inner cell mass as well as in the mural trophectoderm (Fig. 1A,B). In summary, the distribution of CSA positive C3H/HeN cells in correctly developed blastocysts seems to depend on the first contact between the 8-cell stage embryos, and is thus random. All aggregates of the strain combination C3H/HeN x C3H/HeN (positive control) were strongly and homogeneously stained (Fig. 1C) and negative control chimeras (C57BL/6N x BALB/cA) were not stained (data not shown). Poorly developed aggregates (e.g. noncompacted or with 2 blastocoels) were excluded from this experiment (Fig. 1D).

172 aggregates of the various strain combinations were transferred into CD-1, or (BALB/cA x C57BL/6N) F1 foster mothers, in order to analyze the cell mixing pattern 7.5 days p.c. – at the late primitive streak stage. These resulted in 70 implantation sites of which 25 contained degenerated embryos (35.7%) and 45 gave rise to morphologically normal embryos. All embryos were dissected and fixed for subsequent analysis at day 7.5.

As demonstrated by C3H-specific immunostaining, all normally developed embryos could be considered as a true chimeras. Control embryos (C57BL/6N x C57BL/6N or BALB/c x BALB/c) showed no positive staining (data not shown).

Results of aggregation and transplantation experiments are summarized in Tables 1 and 2, respectively.

As shown in Table 3, there was a significantly higher contribution of C57BL/6N cells in 82.6% of C3H/HeN x C57BL/6N chimeras. Estimated proportions of positive (C3H/HeN) cells did not exceed 20% and were located in small distinct clusters (Fig. 2A,B). Only in 2 chimeras (8.7%) was the number of C3H/HeN cells higher than C57BL/6N cells and 2 other chimeras had approximately equal contributions from both genotypes. In all chimeras analyzed, differences in the distribution of CSA positive areas were seen, but no specific and repeatable pattern was obvious. However, in 3 chimeras where C3H/HeN cells repre-

TABLE 3

CELL MIXING IN THE 7.5 DAY OLD CHIMERAS

Mouse strain combination	No. of chimeras evaluated	No. of chimeras showed both genotypes proportionally		No. of chimeras significantly shifted to* C3H/HeH $H\text{-}2^k$		C57BL/6N $H\text{-}2^b$		BALB/cA $H\text{-}2^d$	
		CD-1	F1	CD-1	F1	CD-1	F1	CD-1	F1
C3H/HeN x C57BL/6N	23	2	-	1	1	16	3	-	-
C3H/HeN x BALB/cA	22	-	-	20	2	-	-	-	-

*The proportion of CSA positive cells (C3H/HeN) was visually estimated. The negative cells were considered as C57BL/6N and BALB/cA, respectively. Note that only embryonal tissue was analyzed in this table. In the 3 chimeras of strain combination C3H/HeN x C57BL/6N where genotype ratio was greatly shifted to the C55BL/6N, extraembryonal ectoplacental cone was found uniformly CSA positive.

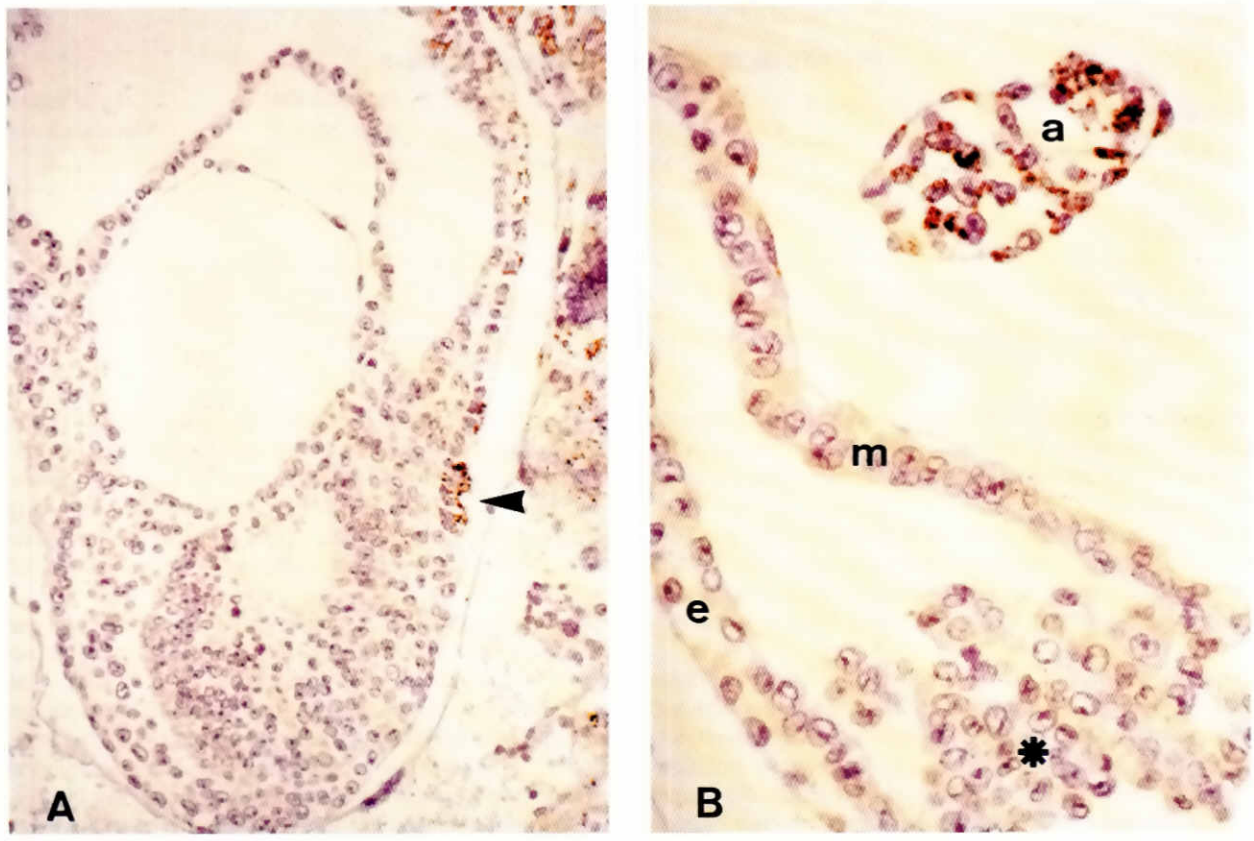

Fig. 2. Immunoperoxidase staining of a C3H/HeN x C57BL/6N chimera at the late primitive streak stage. CD-1 foster mother. Saggital section. (A) *CSA positive cells (arrowhead) are located along the visceral embryonal endoderm. In this case, the ectoplacental cone was positive. Note that CD-1 used as foster mother is a CSA positive crossbreed.* **(B)** *Detail of nonstained embryonal mesoderm (asterisk), extraembryonal endodermal (e) and mesodermal (m) components of visceral yolk sac and positively stained allantois (a) of the same mouse strain combination. Positive cells (C3H/HeN) located only in the allantois and negative cells (C57BL/6N) are clearly distinguishable.*

sented only a minor population in embryonal tissues, extraembryonal ectoplacental cone was found to be uniformly positive (Fig. 3).

In contrast, the second combination of aggregation chimeras – C3H/HeN x BALB/cA clearly showed reduced numbers of BALB/cA cells (CSA negative). In all cases, the estimated contribution of C3H/HeN cells was higher than those of BALB/cA and no exceptions were observed (Fig. 4A). Also contrary to the first strain combination, cells of both genotypes were intermingled in the majority of embryonal and extraembryonal tissues, even though the total number of the C3H/HeN cells was demonstrably higher (Fig. 4B,C).

In addition, implantation sites containing embryos at differing levels of degeneration showed common symptoms of an immunological reaction, such as infiltration by leukocytes (Fig. 5A,B). There was no noticeable tendency to reject the particular strain combination (see Table 2). Also, the ratio of rejected and normal embryos in the CD-1 and (BALB/cA x C57BL/6N) F1 foster mothers was similar, although transplantation into F1 foster mothers generally resulted in lower numbers of implantation sites relative to the total number of embryos transferred. In CD-1, 47.7% of transferred embryos were implanted, whereas only 17.5% of all transferred chimeras were implanted into (BALB/cAxC57BL/6N) F1 pseudopregnant females (see Table 2).

Taken together, these data indicate that cell ratios in aggregation chimeras are initially almost equal and both genotypes are randomly distributed, but that later on, after implantation into the uterus of the foster mother, the relative proportions of different strain derived cells changes dramatically. This haplotype selection is most probably strain combination-specific and the important role of the pseudo-mother's immune system cannot be excluded.

Discussion

The aim of our study was to investigate: i) whether any noticeable tendencies can be seen in cell ratios and cell distribution patterns during the pre- and post-implantation development of aggregation chimeras; ii) whether repeatable cell mixing patterns exist in the two different H-2 haplotype combinations at the blastocyst and primitive streak stages. For this purpose two developmental stages – expanded blastocysts prior to transplantation and late primitive streak fetuses, as well as two different inbred mouse strain combinations, were examined using a strain-specific marker.

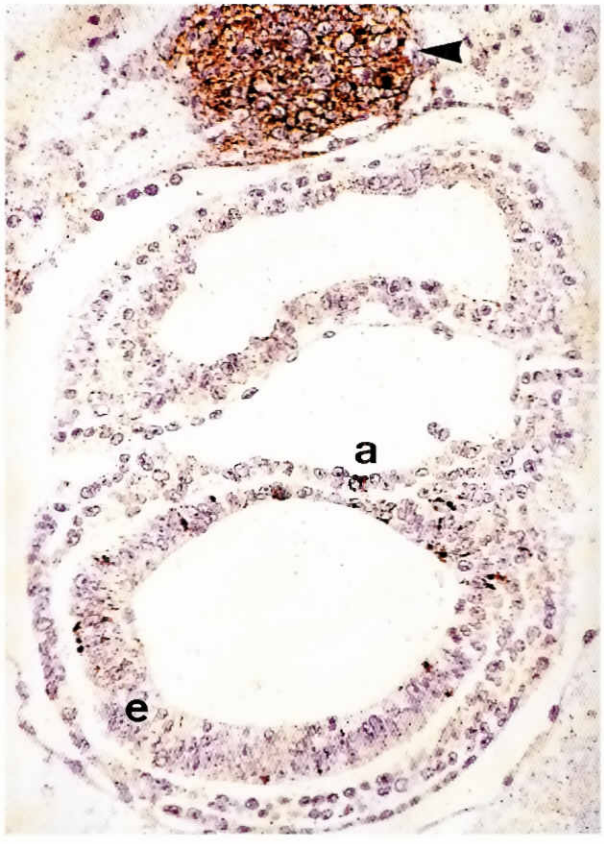

Fig. 3. Immunoperoxidase staining of a C3H/HeN x C57BL/6N chimera at the late primitive streak stage. *CD-1 foster mother is CSA positive. Saggital section. Positive C3H cells are sporadically distributed over the embryonal ectoderm (e) and in the mesodermal component of amnion (a) but the extraembryonal ectoplacental cone is uniformly positive (arrowhead).*

Previous chimeric studies have shown that intensive cell mixing in C3H/HeN x BALB/cA aggregation chimeras occurs early in development – before day 7.5 p.c. (Yoshiki *et al.*, 1993), but no comparative study combining two developmental stages – pre- and post-implantation and the possible role of different H-2 haplotype combinations, has been published.

The immunohistochemical detection of the CSA cell marker allows direct *in situ* localization of individual positive cells and thus offers an ideal system for such studies (Dvorak *et al.*, 1995). We have shown that dramatic changes of the cell ratio in aggregation chimeras, depending on the mouse strain used, occur during the days following transplantation into foster mothers. Because no considerable developmental retardation of any mouse strains used in this study was observed *in vitro* until the expanded blastocyst stage, we hypothesize that pseudomaternal – fetal immune interactions after transplantation are involved in subsequent cell ratio changes.

In addition, we have demonstrated relatively high differences between total numbers of embryos implanted and the number of normally developed embryos at 7.5 days p.c. Also, well implanted but subsequently degenerated embryos showed an advanced state of "rejection" at day 7.5. Thus, one can conclude that certain developmental blocks exist depending on specific immune interactions between the aggregation chimera graft and the host foster mother. It is not clear whether the mechanisms of cell selection or rejection of already implanted embryos could be directly initiated by MHC class I antigens expressed on the embryonal graft or on the maternal trophoblast (Drezen *et al.*, 1994) because functionality of surface H-2 molecules in the mouse placenta remains hypothetical. This means that the presence of MHC class I antigen-specific antibodies in pregnant females of most mammalian species has been described, H-2 antigens on preimplantation mouse embryos can be induced *in vitro* (Warner *et al.*, 1993), but no cellular immune response responsible for tissue rejection has been documented. On the other hand, cytotoxic T lymphocytes were found in some non-lymphoid tissues including the placenta of CBA x DBA/2 mice, which exhibit high rates of spontaneous abortion. Spontaneous fetal rejection in this specific strain combination can be reduced by immunization with allogeneic splenocytes (Chaouat *et al.*, 1985). Similarly, fetal growth and survival was affected either positively or negatively by different cytokines (Chaouat *et al.*, 1990). Finally, Wood (1994) suggested that successful survival of partially MHC incompatible fetuses is mainly caused by restricted antigen presentation, which prevents generation of cell mediated immunity. Unfortunately, in our case it remains unclear what could be the primary signal in the observed phenomenon of "implanted embryonal graft rejection" and resorption. There are at least two possibilities. It may be due to overstimulation of the foster mother's non-adapted immune system by fully healthy and normally developed embryos expressing embryonal H-2 antigens, or by already developmentally damaged conceptuses, which can act as real antigens.

We cannot exclude further organ-specific changes of cell ratio later during development after day 7.5, as Musci and Mullen (1992) have described variations in the spinal cord of BALB/c x C3H/HeN aggregation chimeras developed to term containing from 6 to 90% of BALB/c cells. This is contrary to our results using the same genotype combination, where the contribution of BALB/cA cells seems to be restricted in all day 7.5 chimeras examined.

Nevertheless, on the basis of our observations we can conclude that the very important period for cell mixing in aggregation chimeras and for survival of the embryonal graft is 3 days following successful implantation.

In conclusion we propose that the lack of natural uterine environmental modulations in foster mothers and possible immune modulation of chimeric conceptuses by *in vitro* culture conditions prior to transplantation, together with the specific combination of H-2 haplotypes, can affect survival or resulting cell ratios in the mouse aggregation chimeras.

Materials and Methods

Mice

Three H-2 incompatible strains were used to study genotypic interactions during early development. The strain combinatory pattern was as follows: C3H/HeN (haplotype H-2^k, CSA positive) aggregated with C57BL/6N (haplotype H-2^b, CSA negative) and/or BALB/cA (haplotype H-2^d, CSA negative). In order to obtain embryos, 5-week-old hormonally untreated females were caged with males of the same strain overnight and checked for vaginal plugs the following morning. This day was counted as day 0 of development. CD-1 (CSA positive outbred) and/or

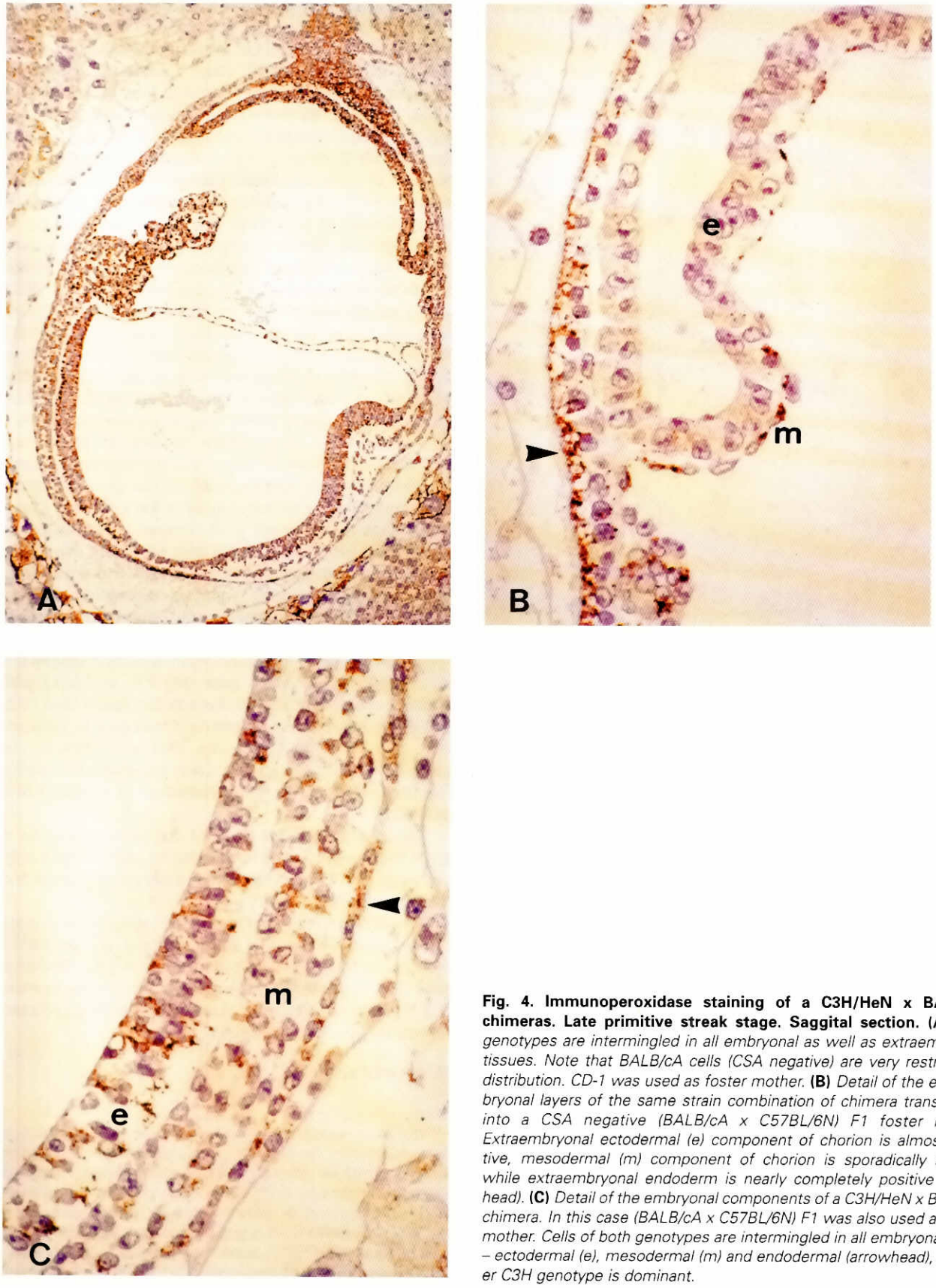

Fig. 4. Immunoperoxidase staining of a C3H/HeN x BALB/cA chimeras. Late primitive streak stage. Saggital section. (A) *Both genotypes are intermingled in all embryonal as well as extraembryonal tissues. Note that BALB/cA cells (CSA negative) are very restricted in distribution. CD-1 was used as foster mother.* **(B)** *Detail of the extraembryonal layers of the same strain combination of chimera transplanted into a CSA negative (BALB/cA x C57BL/6N) F1 foster mother. Extraembryonal ectodermal (e) component of chorion is almost negative, mesodermal (m) component of chorion is sporadically stained, while extraembryonal endoderm is nearly completely positive (arrowhead).* **(C)** *Detail of the embryonal components of a C3H/HeN x BALB/cA chimera. In this case (BALB/cA x C57BL/6N) F1 was also used as foster mother. Cells of both genotypes are intermingled in all embryonal layers – ectodermal (e), mesodermal (m) and endodermal (arrowhead), however C3H genotype is dominant.*

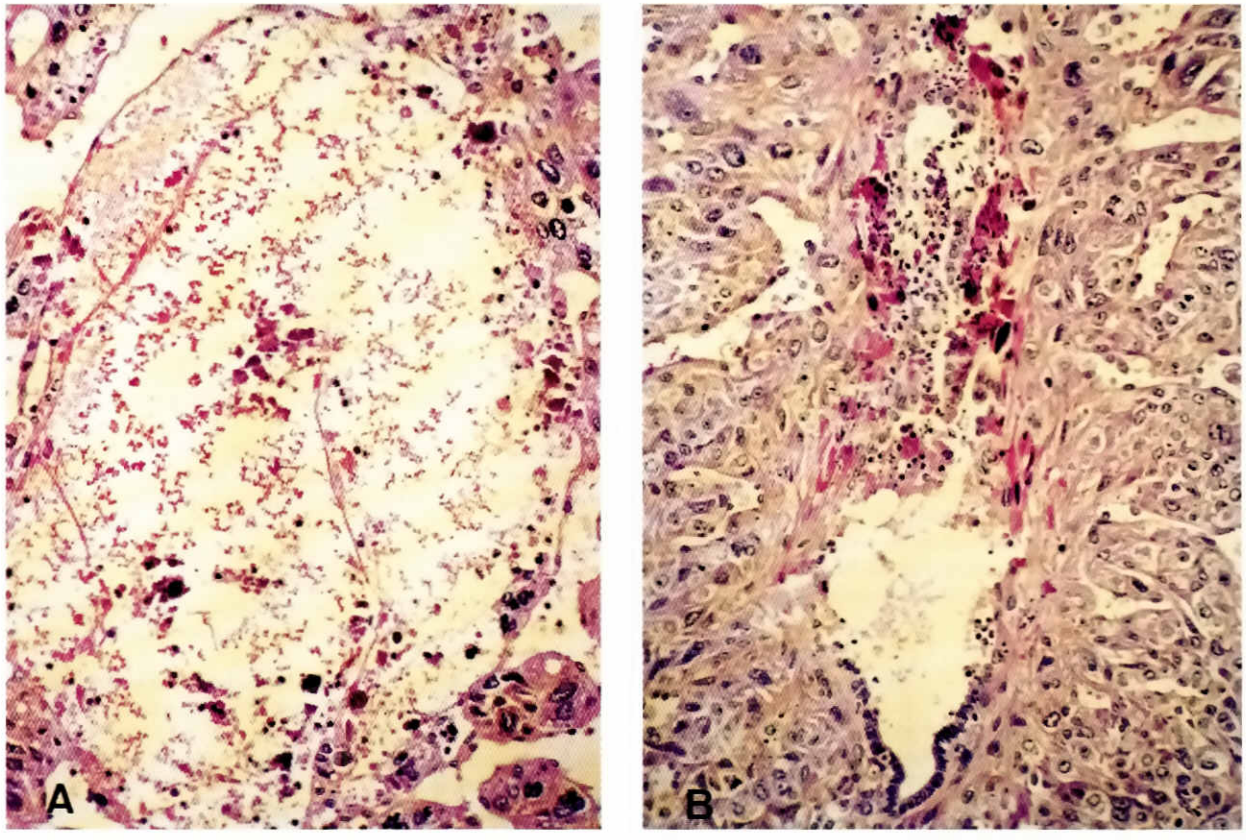

Fig. 5. Decidual tissue with a "rejected" C3H/HeN x C57BL/6N (A) and C3H/HeN x BALB/cA (B) embryo at day 7.5 of gestation. *Both mouse strain combinations were transplanted into a CD-1 foster mother. The concentration of leukocytes is high around the site of implantation. Stained by hematoxylin and eosin.*

(BALB/cA x C57BL/6N) F1 mice were used as foster mothers for studies of embryo-maternal interactions. Mice were maintained in the pathogen free animal facility at the Institute of Physical and Chemical Research, RIKEN, under a constant light-dark cycle (12 h light, 12 h dark) and a standard diet.

Specificity of the anti-CSA antibody

Preparation of mouse hybridoma cell lines secreting antibody against the mitochondrial protein CSA has been reported previously (Kusakabe *et al.*, 1988). This monoclonal antibody recognizes an antigenic epitope which is controlled by the CSA gene region containing a single base substitution (Michikawa *et al.*, 1993). Of all inbred mouse strains used for aggregation, only the C3H/HeN strain has this point mutation yielding a detectable CSA.

Aggregation chimeras and embryo-transfer

8-cell embryos were recovered by flushing of oviducts on day 2 of development. The zona pellucida was removed by pronase digestion (Boehringer Manheim) diluted in M 2 medium. Only morphologically equivalent embryos were selected for aggregation. Pairs of zona-free embryos, one of each genotype, were aggregated in drops of previously equilibrated M 16 medium and cultured overnight. For optimal cell to cell contact, aggregates were placed into previously prepared microholes. Alternatively, an aggregation technique using phytohemagglutinin-P (5 µl/ml of M 2 media, Difco, Detroit, MI, USA) was used. After overnight culture, all drops containing aggregates were carefully examined for the presence of degenerated or free blastomeres and some of the embryos that had developed correctly into single blastocysts were used for transfer experiments. Usually, 8-10 aggregates of one strain combination were transferred into the right uterine horn and same number of the second combination of chimeras into the left uterine horn. Day 2 p.c. pseudo-pregnant recipients were used. Remaining non-transferred blastocysts were fixed and stained by anti-CSA antibody for cell mixing analysis at the blastocyst stage. CSA negative control aggregates were produced by combination of embryos of same H-2 haplotype, C57BL/6N or BALB/cA.

Fixation and staining of chimeras

Two protocols were used for fixation of blastocysts and 7.5 day-old embryos, respectively. Blastocysts were fixed 1 h in ice cold 95% ethanol containing 1% acetic acid, post-fixed 30 min in ice cold ethanol and rehydrated slowly (95%, 90%, 80%, 70% and 50% ethanol, followed by distilled water and PBS; each for 5 min).

The protocol described in detail by Yoshiki *et al.* (1993) was carried out for fixation of 7.5-day-old chimeras. Briefly, pregnant recipients were sacrificed and their uterine horns were rapidly dissected into PBS, then transferred to 0.1 M Na-phosphate buffer (pH 7.4) and pre-fixed 20 min by microwave irradiation (Bio-Rad H 2500 microwave processor). Pre-fixed embryos with uterus and decidual swelling were fixed overnight at 4°C in 95% ethanol, 1% acetic acid, dehydrated by ethanol and embedded individually in polyester wax. 4 µm thick sections were cut and mounted on egg white coated slides. A total of 50-100 slides were prepared from each chimera, 5-10 representative saggital sections were selected by hematoxylin/eosin staining of every 10th slide, dewaxed and rehydrated before processing for immunocytochemistry.

For immunostaining of entire blastocysts, embryos were pre-treated 1 h in PBS containing 5% normal goat sera (NGS), 1% BSA and incu-

bated overnight in anti-CSA antibody. After washing in PBS, the blastocysts were incubated 1 h in goat anti-mouse IgG(H+L)-FITC-conjugated second antibody (Zymed, Burlingame, CA, USA) and transferred to the fluorescence preventing mounting fluid (Difco, Detroit, MI, USA).

Immunostaining of sections was carried out according to Yoshiki *et al.* (1993). Briefly, non-specific endogenous peroxidase activity was inactivated in methanol containing H_2O_2 and sodium azide and slides were subsequently incubated in avidin D and biotin blocking solutions (Vector, Burlingame, CA, USA). Sections were then incubated overnight in biotinylated anti-CSA antibody, washed and incubated with peroxidase-conjugated avidin (Zymed, Burlingame, CA, USA). The peroxidase substrate solution used was 0.05 M Tris-HCl containing 0.27 mg/ml 3,-3'diaminobenzidine (Dojin, Kumamoto, Japan) and 0.0088% H_2O_2. The enzymatic reaction was enhanced by silver/gold, slides were counterstained with hematoxylin and eosin and mounted in Malinol (Muto Pure Chemical, Tokyo, Japan). Sections were examined and photographed using a Nikon Microphot-FXA light microscope.

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References

BARNES, R.D. (1976). The use of early embryo aggregation derived mouse chimaeras. III. A tool of immunogenetics. *J. Immunogenet. 3:* 423-428.

BARSH, G.S., LOVETT, M. and EPSTEIN, C.J. (1990). Effects of the lethal yellow (Ay) mutation in mouse aggregation chimeras. *Development 109:* 683-690.

BILLINGHAM, R.E. (1964). Transplantation immunity and the maternal-fetal relation. *New Engl. J. Med. 270:* 667-672.

CHAOUAT, G., KOLB, J-P., KIGER, N., STANISLAWSKI, M. and WEGMANN, T.G. (1985). Immunologic consequences of vaccination against abortion in mice. *J. Immunol. 134:* 1594-1598.

CHAOUAT, G., MENU, E., CLARK, D.A., DY, M., MINKOWSKI, M. and WEGMANN, T.G. (1990). Control of fetal survival in CBA x DBA/2 mice by lymphokine therapy. *J. Reprod. Fertil. 89:* 447-458.

CRANDALL, J.E. and HERRUP, K. (1990). Patterns of cell lineage in the cerebral cortex reveal evidence for developmental boundaries. *Exp. Neurol. 109:* 131-139.

CRUSIO, W.E., BAR, I.M., SCHWEGLER, H. and BUSELMAIER, W. (1990). A multivariate morphometric analysis of hippocampal anatomical variation in C57BL/6 in equilibrium BALB/c chimeric mice. *Brain Res. 535:* 343-346.

DAVID-WATINE, B., TRANSY, C., GACHELIN, G. and KOURILSKY, P. (1987). Tissue-specific expression of the mouse Q10 H-2 class-I gene during embryogenesis. *Gene 61:* 145-154.

DELTOUR, L., LEDUQUE, P., PALDI, A., RIPOCHE, M.A., DUBOIS, P. and JAMI, J. (1991). Polyclonal origin of pancreatic islets in aggregation mouse chimeras. *Development 112:* 1115-1121.

DREZEN, J-M., BARRA, J., BABINET, C. and MORELLO, D. (1994). MHC class I genes are not imprinted in the mouse placenta. *Immunogenetics 40:* 62-65.

DREZEN, J-M., NOUVEL, P., BABINET, C. and MORELLO, D. (1992). Different regulation of class I gene expression in the adult mouse and during development. *J. Immunol. 149:* 429-437.

DVORAK, P., DVORAKOVA, D., YOSHIKI, A., OHASHI, T., KITAMURA, K. and KUSAKABE, M. (1995). Expression of paternal and maternal mitochondrial HSP 70 family, hsc 74, in preimplantation mouse embryos. *Int. J. Dev. Biol. 39:* 511-517.

FUNDELE, R., BOBER, E., ARNOLD, H.H., GRIM, M., BENDER, R., WILTING, J. and CHRIST, B. (1994). Early skeletal muscle development proceeds normally in parthenogenetic mouse embryos. *Dev. Biol. 161:* 30-36.

HEDLEY, M.L., DRAKE, B.L., HEAD, J.R., TUCKER, P.W. and FORMAN, J. (1989). Differential expression of the class I MHC genes in the embryo and placenta during midgestational development in the mouse. *J. Immunol. 142:* 4046-4053.

HUNT, J.S. and ORR, H.T. (1992). HLA and maternal-fetal recognition. *FASEB J. 6:* 2344-2348.

JÄGERBAUER, E-M., FRASER, A., HERBST, E.W., KOTHARY, R. and FUNDELE, R. (1992). Parthenogenetic stem cells in postnatal mouse chimeras. *Development 116:* 95-102.

KANBOUR-SHAKIR, A., ZHANG, X., ROLEAU, A., ARMSTRONG, D.T., KUNZ, H.W., MacPHERSON, T.A. and GILL, T.J. (1990). Gene imprinting and major histocompatibility complex class I antigen expression in the rat placenta. *Proc. Natl. Acad. Sci. USA 87:* 444-448.

KUSAKABE, M., YOKOYAMA, M., SAKAKURA, T., NOMURA, T., HOSICK, H.L. and NISHIZUKA, Y. (1988). A novel methodology for analysis of cell distribution in chimeric mouse organs using a strain specific antibody. *J. Cell Biol. 107:* 257-265.

KYDD, J.H., BUTCHER, G.W., ANTCZAK, D.F. and ALLEN, W.R. (1991). Expression of major histocompatibility complex (MHC) class I molecules on early trophoblast. *J. Reprod. Fertil. (Suppl.) 44:* 463-477.

MATSUNAGA, T., SIMPSON, E. and MEO, T. (1980). Allogeneic tolerance in embryo aggregation mouse chimeras studied by mixed lymphocyte culture and cell-mediated lympholysis. *Transplantation 30:* 34-39.

MATTA, C.A. (1991). Formation by aggregation of viable chimeras between ICM and eight-cell mouse embryos with different genetic backgrounds. *Funct. Dev. Morphol. 1:* 35-39.

MICHIKAWA, Y., BABA, T., ARAI, Y., SAKAKURA, T. and KUSAKABE, M. (1993). Structure and organization of the gene encoding a mouse mitochondrial stress-70 protein. *FEBS Lett. 1:* 27-33.

MUSCI, T.S. and MULLEN, R.J. (1992). Cell mixing in the spinal cords of mouse chimeras. *Dev. Biol. 152:* 133-144.

NAGY, A., GOCZA, E., DIAZ, E.M., PRIDEAUX, V.R., IVANYI, E., MARKKULA, M. and ROSSANT, J. (1990). Embryonic stem cells alone are able to support fetal development in the mouse. *Development 110:* 815-821.

PHILPOTT, K.L., RASTAN, S., BROWN, S. and MELLOR, A.L. (1988). Expression of H-2 class I genes in murine extra-embryonic tissues. *Immunology 64:* 479-485.

SEPULVEDA, S. and IZQUIERDO, L. (1990). Effect of cell contact on regionalization of mouse embryos. *Dev. Biol. 139:* 363-369.

SILVERMAN, T., REIN, A., ORRISON, B., LANGLOSS, J., BRATTHAUER, G., MIYAZAKI, J-I. and OZATO, K. (1988). Establishment of cell lines from somite stage mouse embryos and expression of major histocompatibility class I genes in these cells. *J. Immunol. 140:* 4378-4387.

TACHI, C., YOKOYAMA, M. and KOJIMA, H. (1990). Analysis of coat-color patterns in aggregation chimeras between BALB/cA and C3H/HeN mice with special reference to migratory patterns and clone number of epidermal melanoblasts. *Dev. Genet. 11:* 254-262.

WARNER, C.M., ALMQUIST, C.D., TOULIMAT, M.H. and XU, Y. (1993). Induction of embryonic major histocompatibility complex antigen expression by gamma-IFN. *J. Reprod. Immunol. 24:* 111-121.

WOOD, G.W. (1994). Is restricted antigen presentation the explanation for fetal allograft survival? *Immunol. Today 15:* 15-18.

YAHATA, G., UEDA, H., TAKAKUWA, K., KODAMA, S., TANAKA, K. and KANAZAWA, K. (1990). Differential expression of human leukocyte antigen-A,-B, and -C locus specific genes in trophoblast and embryonic cells. *Cell Growth Differ. 1:* 73-78.

YOSHIKI, A., SAKAKURA, T. and KUSAKABE, M. (1993). The mouse chimera during intrauterine stages: immunohistochemical analysis with the C3H strain-specific antibody. *J. Histochem. Cytochem. 41:* 1583-1590.

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