Chimerism in health, transplantation and autoimmunity

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1. Historical background

Mythology

The term 'chimerism' originates from Greek mythology and refers to the creature Chimaera (in Greek Χίμαιρά). Chimaera had a monstrous appearance and is mentioned in the poems of many great ancient writers, such as Homer, Vergil, and Ovid. Homer described Chimaera as follows: "she was of devine stock, not of men, in front a lion, in back a serpent, and in the middle a goat, breathing out terribly the force of blazing fire."[1] (Figure 1). Although other descriptions of Chimaera's anatomy exist, she is always a fire-breathing fusion of parts derived from a lion, a goat, and a snake or dragon. The goat anatomy gives the creature its name, since Chimaera literally means 'goat' in ancient Greek. Chimaera came forth out of a monstrous family consisting of many notorious creatures.

Figure 1. 'La Chimera di Arezzo'. The mythological Chimaera is a creature consisting of tissues from several different species. This is an Etruscian bronze sculpture created in the 4th century BC located in the Museo Archeologico Nazionale in Florence, Italy.

Over time, Chimaera has been a popular subject in art as a fantastic symbol. However, it was not only the monstrous nature of her anatomy that made her immortal over time, but also the fact that her body consists of parts derived from three completely different animals that exist and function 'peacefully' together. It is this last characteristic that

scientists refer to when they speak of a chimeric organism, a single organism composed of at least two genetically different types of tissue. Similarly, in medicine, the term chimerism is used to refer to an individual, organ, or part consisting of tissues of diverse genetic constitution.[2]

Definition of chimerism in medicine

The definition of a chimaera in medicine, where it is usually referred to as chimera, was first described in detail by Ford in 1969.[3] He based his definition on previous articles and stated it as follows: a chimera is an organism whose cells are derived from two or more distinct zygote lineages. Therefore, he distinguishes a chimera from a mosaic, in which there are also two or more chromosomally distinct cell lines present in one individual, but, in contrast to a chimera, a mosaic is formed by the cells of a single zygote lineage.

Furthermore, Ford distinguished two hypothetical groups of human chimeras: 1) those originating through two separate acts of syngamy in one ovum and 2) those originating from the cells of two independent zygotes. The first group mainly involves dispermy leading to two fertilizations in one ovum. Also, the fusion of the zygote nucleus with the nucleus of the second polar body may lead to chimerism. Both possibilities would always result in chromosome number abnormalities. The second group of chimerism is better known and involves the early fusion of two embryos, placental cross-circulation between dizygotic twins, maternal-fetal transplacental exchange, and artificial chimerism due to transplantation or transfusion.[3]

Historical background of chimerism in medicine

To our knowledge, the first report of chimerism in medicine was by Schmorl in 1893.[4] He performed autopsies on 17 pregnant women who died of eclampsia.[5] In the lung capillaries of these women he found thrombi containing multi-nucleated syncytial trophoblast cells of fetal origin (Figure 2). With this discovery of fetus-derived cells in the maternal circulation, Schmorl was the first to suggest the occurrence of fetal-maternal cellular trafficking during pregnancy, although the situation he described was not physiological.

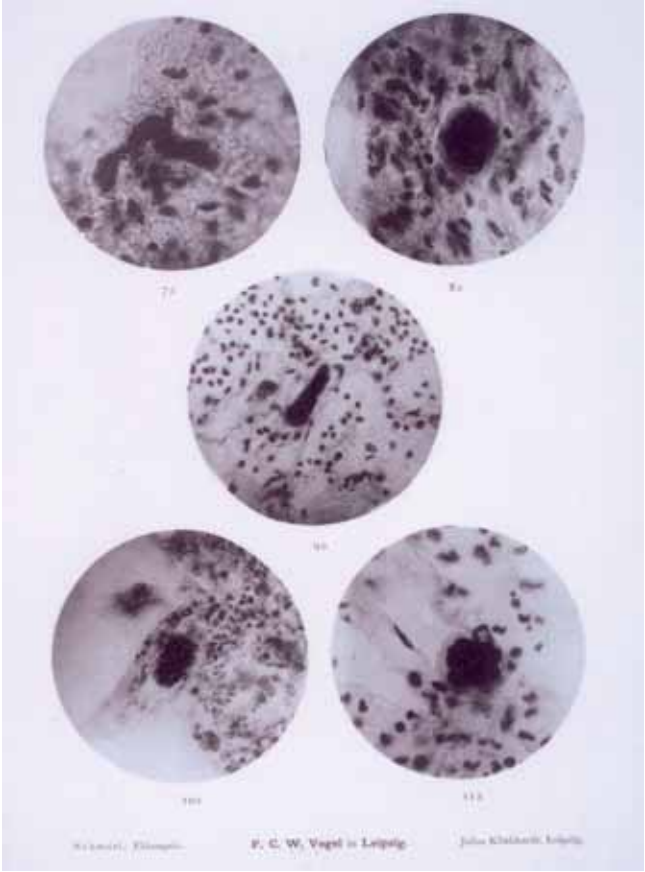

Figure 2. Trophoblast cells in the lung capillaries of women who died of eclampsia as described by Schmorl in 1893.[5] Leiden University Library (reference 1363 C30).

It was the fetal-fetal cellular trafficking between twins that led to more discoveries in the field of chimerism. In 1916, Lillie[6] was fascinated by the fact that, in the case of bovine twins of different sex, the female bovine was frequently born sterile (also called a Freemartin). He suggested that if one twin is male and the other female, the reproductive system of the female is suppressed by hormones from the male. This hypothesis was supported by the finding that the choria of bovine twins can fuse in utero, and blood vessels from the zygotes can anastomose in the connecting part of the two choria leading to a constant interchange of blood between the twins. With the demonstration of the interchange of blood between twins, it seemed very likely that

these twins were chimeric for each other's blood cells. This was confirmed by Owen in 1945 by demonstrating that, in dizygotic bovine twins, a mixture of two distinct types of erythrocytes can be found, one that is genetically their own and the other belonging to their twin.[7] Interestingly, this blood group chimerism was also detected in the blood of adult cattle. Therefore, Owen concluded that these chimeric cells are apparently capable of becoming established in the hematopoetic tissues of their co-twin and continue to provide a source of blood cells distinct from the host, presumably throughout life. This was the first time that a 'stem cell-like nature' of chimeric cells was suggested.

Evidence for blood chimerism was found not only in animals, but also in humans. In 1953, Dunsford et al.[8] reported the case of a 25-year-old female blood donor whose blood grouped as a mixture of blood group A and O (39% and 61% of the blood cells, respectively). The woman had never had a blood transfusion. Remembering the paper by Owen,[7] the investigators asked whether this woman was a twin, and it appeared that her twin brother had died 25 years prior at the age of 3 months. The uniqueness of this situation was underlined by earlier investigations of the Blood Group Research Unit on the blood of 58 pairs of dissimilar twins and 82 pairs of apparently identical twins in which no evidence of blood group chimerism was found.[8] In 1957, Booth et al.[9] presented another example of a pair of dizygotic twins in which the twin brother had red blood cells that were 86% blood group A (genetically his own) and 14% blood group O (genetically from his sister). His twin sister also had a mixture of blood group A and O, though 1% and 99%, respectively. In the same issue of the British Medical Journal, another case of human twins chimeric for each other's red and white blood cells was presented.[10]

Apart from the chimerism detected as two different blood group types of twins, chimerism has been found frequently in pregnant women as demonstrated by the presence of fetal cells in maternal blood. In 1969, Walknowska et al.[11] collected peripheral blood from 30 healthy pregnant women and investigated if cells with a 46/XY karyotype were present. They found them in 21 women, of whom 19 were pregnant with a male child, suggesting that the male cells found in these women were fetus-derived. Similar

results were found in other studies.[12,13] Before 1996, studies addressing the presence of chimerism as a result of maternal-fetal transplacental exchange mainly investigated material from pregnant women. However, in a 1996 landmark study, Bianchi et al.[14] described an example of male cells in a woman who was not pregnant at that time but had given birth to a son 27 years before. They concluded that chimeric cells derived from pregnancies can persist in the host for years, in line with Owens findings in cattle.

Immunological consequences of chimerism

Since the first discovery of chimeric cells in individuals, investigators have been intrigued by the immunological consequences, especially as to why these genetically foreign cells are tolerated by the host. As early as 1914, Murphy showed that rat tissue could grow on the chorioallantoic membrane of chick embryos without being rejected,[15] a finding that could not be repeated in adult tissue. When the rat tissue was grafted to the embryo together with the spleen tissue or bone marrow from adult chickens, the rat tissue did not survive. Other tissues from the adult chicken, such as the kidney and liver, did not have this effect, demonstrating that the active immunological capacities of cells lie in the adult bone marrow and spleen. This was one of the first examples of how embryos can become tolerant to completely foreign (i.e. allogenic) cells.

Medawar and his colleagues went a step further by demonstrating that tolerance to antigens encountered before birth can persist into adult life.[16,17] They elaborated on the Freemartin phenomenon and the need for farmers to distinguish monozygotic and dizygotic cattle twins. They hypothesized that only monozygotic twins, being genetically similar, would be tolerant to each other's skin grafts. For dizygotic twins, the situation would be comparable to that of full siblings of separate birth, for which it was already known that they do not tolerate each other's skin grafts. However, to their surprise, they found that out of 42 individual cattle that received skin grafts from their respective dizygotic twins, 36 (86%) proved to be completely tolerant. Referring to the work of Owen, they concluded the following: "the work of Owen justifies the strong presumption that tolerance to skin grafts exchanged between two-egg twins is a consequence of the same peculiarity of embryonic development as that which leads to sexual abnormality in the female member of two-egg twin pairs of unlike sex: the

anastomosis of the fetal circulations."

In humans, the same experiment was performed with the twins described by Booth et al.[9] as blood group chimeras.[18] Both twins (a male and a female) gave consent to receive a skin transplant from their twin sibling on the volar site of their wrist. The male skin transplanted to the female twin was completely tolerated and was still male. The female skin transplanted to the male twin was also tolerated, and it appeared that almost all cells of the transplant had become male. This was the first example in humans that tolerance for each other's grafts exists between chimeras. Again it was hypothesized that this tolerance was acquired during embryological exposure to antigens of the twin sibling.

The interpretation that tolerance for foreign grafts can be acquired if the host has been exposed to the antigens of the donor sufficiently early in fetal life was later verified in mice by Billingham, Brent, and Medawar.[19] In this study, six fetuses from a CBA mouse mother were intra-embryonically injected with 0.01 ml of a suspension of adult tissue from an adult A-line donor. Five neonates were born and, after 8 weeks, received a skin transplant from the adult A-line donor. In two mice the grafts were quickly rejected, in one mouse a delayed graft rejection occurred after 91 days, and the other two mice remained completely tolerant. The authors concluded that the mice had acquired tolerance for cells from the A-line donor by intra-embryonic exposure to these cells. Embryonic actively-acquired tolerance was also demonstrated in chickens by Milan Hašek.[20]

The findings by Medawar et al.[19] relate to the question scientists had been fascinated by since the start of the 20th century, namely, how tolerance is established to self-antigens and thereby prevents autoimmunity from occurring. It is, therefore, not surprising that when Burnet formulated his clonal selection theory in 1957,[21] the tolerance to antigens experienced by an individual in embryonic life formed an important argument in favor of his theory. The clonal selection theory formulated by Burnet states that a particular lymphocyte is selected by antigen and then proliferates, resulting in clones of daughter cells producing antibodies with the same specificity. According to this theory, the entire immune repertoire is generated in early embryonic development, and

antigens encountered before birth result in the deletion of the clones specific for them (termed 'forbidden clones'). Antigens encountered after birth activate specific clones of lymphocytes to produce antibodies, of which, the specificity is encoded in the genome of the antibody-producing cell.

Recapitulating the data and the theories of Medawar and Burnet, it can be concluded that chimerism induced during embryonic life leads to acquired tolerance for the foreign chimeric antigens, a process that may be identical to the process of natural tolerance because self-antigens are also 'seen' during embryonic life. The fact that Owen's chimerism experiments in cattle twins formed the basis of Medawar and Burnet's work, for which they received a shared Nobel prize in 1960, was underlined in the letter Medawar wrote to Owen; in the letter he stated that Owen should have shared in the Nobel prize as well because his experiments, as Medawar states it, 'started it all'.[22]

Later, experiments demonstrated that tolerance can also be established after birth, the neonate is in fact immunocompetent, one plasma cell can produce different antibodies, and the specificity of antibodies is not encoded in our genome as that would require much more genes than we actually have, thereby weakening Burnet's theory of natural tolerance. Although we now know much more about tolerance than Burnet and others did at that time, scientists still have not answered the questions of why we are tolerant to self-antigens, why chimeric cells are tolerated by the host, and why autoimmunity sometimes occurs.

2. Sources of chimeric cells

Below, we will discuss three important sources of chimeric cells, namely: chimeric cells derived from pregnancies, blood transfusion, and transplantation. Pregnancy may lead to chimerism in several ways, and a variety of cell types can be transferred during pregnancy. We will also pay attention to the role of chimerism for prenatal diagnostics. Blood transfusion would seem to be the most obvious way to induce chimerism. We will discuss some interesting results from several studies investigating the fate of chimeric

cells after blood transfusion. Transplantation encompasses solid organ and bone marrow transplantation, both of which may lead to chimerism, though via different routes.

Pregnancy

The fetal circulation is separated from the maternal circulation by the placental barrier, allowing the exchange of metabolic and gaseous products. The basic idea of a placental barrier was already presented in the 18th century by John and William Hunter who injected liquid wax into the uterine artery and discovered that the wax did not appear in the fetal circulation.[23] The placental barrier prevents a large intermingling of fetal and maternal blood, but does not maintain absolute integrity and small amounts of fetal blood, and therefore fetal cells, may enter the maternal circulation leading to chimerism.

Development of the placenta and its circulation

After fertilization of the ovum in the fallopian tube, the formed zygote undergoes a series of rapid mitotic cell divisions known as cleavage, after which it is called the blastomere. Approximately three days after fertilization, subsequent cell divisions of the blastomere result in the formation of the morula, which enters the uterine cavity. At day 4, the morula is converted into a blastocyst that consists of an inner cell mass, the embryoblast, which will form the embryo; a blastocyst cavity; and an outer cell layer, the trophoblast, which will form the embryonic part of the placenta. The blastocyst attaches to the endometrium four to five days after fertilization. At that point, the villous trophoblast layer differentiates into the cytotrophoblast and the syncytiotrophoblast. The former is mitotically active, the latter rapidly transforms into a large multinucleated mass without distinguishable cell membranes. The syncytiotrophoblast invades the maternal epithelium and underlying stroma and, six or seven days after fertilization, the blastocyst is superficially implanted. The functional layer of the endometrium in pregnancy is called the decidua, which is the maternal component of the placenta. In the second week of human development, a lacunar network is formed in the syncytiotrophoblast and the opening of uterine vessels into these lacunae establishes the beginning of the uteroplacental circulation. At the end of the second week, the primary chorionic villi are formed. The fetal component of the placenta is formed by the chorionic plate, from which the chorionic villi arise. Maturation of the villous tree

into secondary and, later, tertiary villi containing chorionic vessels connecting to the embryonic circulation results in a primitive fetoplacental circulation by the end of the third week of embryonic development.[24,25]

The large surface area of the chorionic villi, which are bathed in maternal blood present in the intervillous space, enables the exchange of oxygen, nutrients, and excretory products between the embryonic and maternal circulation. The two circulations are separated by the so-called placental barrier, which consists of five layers (Figure 3).[26] Starting from the maternal side: 1) a continuous layer of syncytiotrophoblast cells, 2) an initially (in the first trimester) complete but in the second and third trimester discontinuous layer of cytotrophoblast cells, 3) a trophoblastic basal lamina, 4) connective tissue derived from the extra-embryonic mesoderm, and 5) the fetal endothelium. Throughout pregnancy, the placental barrier becomes progressively thinner while fetal blood flow and brood pressure increase as the villous tree enlarges.[27] Particularly in the third trimester, small microscopic disruptions of the placental barrier allow fetal cells to leak into the intervillous space and, thereby, enter the maternal circulation. However, there is no gross intermingling of the macromolecular constituents of the two circulations.

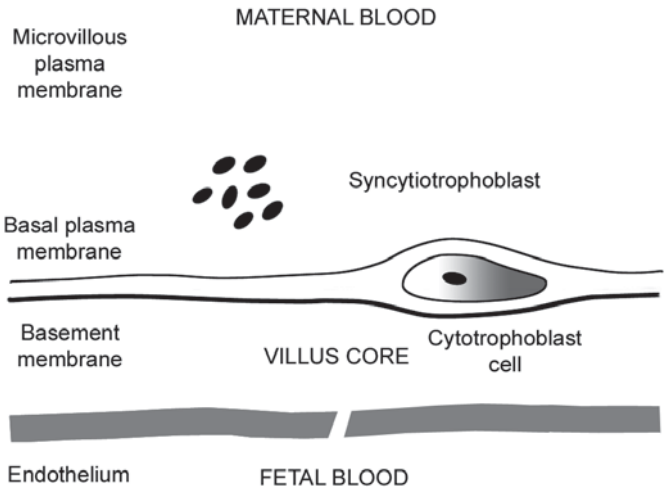

Figure 3. The major components of the placental barrier between maternal and fetal blood near term. From Glazier et al.,[26] with permission.

Iceberg of pregnancy

Healthy couples having intercourse regularly without contraception have a 25 to 30 percent chance of beginning a recognized pregnancy in a given menstrual cycle.[28-30] Ten to 20 percent of these pregnancies are subsequently lost and spontaneous abortions account for almost all of these losses.[29,31,32] Wang et al.[29] studied 518 healthy Chinese women for 12 months and detected 434 clinical pregnancies (defined as any pregnancy that lasted ≥6 weeks after the onset of the last menstrual period and was confirmed by hCG assay). Of these pregnancies, 373 ended as live birth (86%), 49 (11%) as spontaneous abortions, six as induced abortion (1.4%), two as ectopic pregnancies or moles (0.46%), and four as still births (0.92%).

When couples do not achieve a clinical pregnancy, it is either because they did not conceive at all, or they conceived, but the pregnancy ended before it was detected clinically. After the introduction of sensitive assays for human choriogonadotropin (hCG), it has become clear that a large number of conceptions fail before a woman becomes aware that she may have been pregnant. In vitro studies have shown that hCG is produced by trophoblastic cells of the unhatched blastocyst and may be detected as early as 7 days after fertilization.[33,34] Wilcox et al.[32] studied 221 women and analyzed hCG in daily urine samples collected during 6 months of attempted conception. Of the 198 pregnancies detected, 31% were lost. Twenty-two percent of these were early pregnancy losses, occurring before a woman could have been aware of the pregnancy. In similar studies, the rates of early pregnancy loss were comparable and ranged from 13 to 37%.[28-31,35] The discrepancies may be partly explained by the different sensitivities of the hCG assays used, differences in study design with respect to characteristics of the women included, and the collection of urine samples. However, taken together, data from the published studies point to a rate of pregnancy loss prior to implantation of 30%, a further 30% following implantation, but prior to the missed period, and 10% as clinical miscarriages (Figure 4).[36] Therefore, the remaining 30% of live births is only the tip of the iceberg. Taken this data into consideration, it is important to realize that not only pregnancies resulting in live births but many other pregnancies, of which many may pass unnoticed, may result in chimerism.

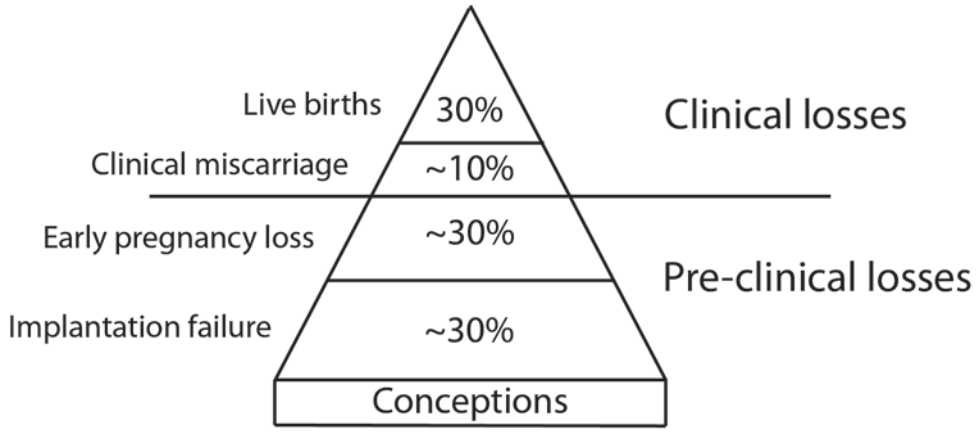

Figure 4. The human pregnancy loss iceberg: an overview of the outcome of spontaneous human pregnancy. A total of 70% of all conceptions are lost prior to live birth. The majority of these losses occurs prior to the time of the missed menstrual period, and is not revealed. (Based on Macklon et al.[36])

Interestingly, it has been reported that women with fertility problems experience a relatively higher number of pregnancies ending in early pregnancy losses than women without fertility problems. Hakim and colleagues[35] studied 124 women, of which 50 had a history of fertility problems classified as a ≥12 month delay of conception or treatment for infertility before entering the study. There were 1.7 pregnancies per woman among subfertile women, compared with 1.4 pregnancies in women without fertility problems. Women with fertility problems had a somewhat higher rate of clinically diagnosed miscarriages but had significantly more early pregnancy losses than women without any evidence of fertility problems (relative risk 2.6, 95% CI 1.8 to 3.8).

The vanishing twin

The prevalence of live-born twins in North America is now 32 in 1000 births.[37] The number of monozygotic twins has been relatively constant. With the increased use of fertility drugs and in vitro fertilization since the 1980s, there has been a pronounced rise in dizygotic twinning rates in live births (e.g. in the US, the twinning rate has climbed 70% in the period of 1980-2004).[37] Similar trends were observed in other countries. Monozygotic twinning entails one zygote splitting into two separate individuals and represents about a third of all spontaneous twins.[38] Because monozygotic twins are,

per definition, genetically identical, twin-derived chimerism can only occur in a dizygotic twin pregnancy.

With the increasing use of ultrasound examinations, especially in the first trimester, researchers have noted that many twin pregnancies are lost or convert to a singleton pregnancy. This is called the 'vanishing twin' phenomenon.[39-44] Landy et al.[39] evaluated 1000 pregnancies by the first trimester ultrasound with a minimum twinning incidence of 3.3%. In 21.2% of these twin pregnancies, the pregnancies ended as singleton births. This process was most often accompanied by vaginal bleeding. If women with a suspect diagnosis of twinning during the ultrasound were included, the incidence of multiple conceptions was 4.99%, and the rate of one disappearing fetus increased to 48%. Similar rates of twin pregnancies resulting in singleton births were reported by other groups.[41,42] Sampson and de Crespigny followed 126 twin pregnancies detected by ultrasound examination at 6-16 weeks of gestation. Of pregnancies with live twins detected prior to 7 weeks of gestation, 29% resulted in the birth of one child. This percentage decreased to 16% for twin pregnancies diagnosed between 7 weeks and 8 weeks 6 days.[42] Other studies have reported higher rates of vanishing twins, ranging from 53 to 71%.[40,43,45] Studies in which a pathological examination of the placenta was performed after delivery are scarce.[46,47] Jauniaux et al.[46] found histological evidence of the vanishing twin phenomenon in five out of 10 placentas from pregnancies with ultrosonographic evidence of a vanished twin. Taken together, it appears that a substantial number of pregnancies that begin as twin pregnancies end as singleton births. Therefore, individuals born as a singleton may be chimeric for their vanished twin.

Chimerism and prenatal diagnostics

Research on chimerism has gained much interest in recent years, especially with respect to its potential use in prenatal diagnostics. Over the past few decades, the utilization of prenatal diagnostics has expanded, primarily due to two trends: smaller family size, with an increased emphasis on assurance of the healthiness of each child, and advancing maternal age. In the Netherlands, it is currently considered 'standard clinical practice' to offer prenatal cytogenetic diagnosis to pregnant women who are 36 years or older at

the 18th week of gestation.[48] These cytogenetic diagnoses are facilitated by obtaining fetal nucleated cells via an invasive technique, such as chorionic villus sampling or amniocentesis. Irrespective of the accuracy of these techniques, the incidence of trisomy 21, the most common autosomal aneuploidy, is still close to 1 per 1000 live births, which is relatively high. An important reason for this is that prenatal diagnostic techniques are directed towards a minority of pregnant women. Although older pregnant women are individually at a higher risk of having a baby with Down syndrome, as a group, they have only a small fraction of the total number of births. Eighty percent of the newborns with trisomy 21 are born to women under age 35 who are not offered the invasive prenatal diagnostic techniques because the risk of a complication following the procedure resulting in fetal loss is higher than the incidence of Down syndrome. Hence, increased attention has been paid to non-invasive techniques for screening fetal trisomy 21 (and other aneuploidies) that can be safely offered to all pregnant women.

Although the transfer of fetal cells into the maternal circulation was already described by Schmorl in 1893, it took nearly a century before concrete evidence of fetomaternal cellular transfer was obtained with the use of molecular techniques like fluorescence in situ hybridization (FISH) or the polymerase chain reaction (PCR). With these techniques, the existence of fetal-specific sequences in maternal blood, for example Y chromosome-associated-sequences, was proven beyond a doubt.[49,50] The successful isolation of fetal cells from maternal blood represents a source of fetal chromosomes and DNA that can be obtained non-invasively by maternal venapuncture. The main advantage of isolated fetal cells is that they offer a pure source of the entire fetal genome without the possible inclusion of maternal genetic material. This is important when examining Mendelian disorders because the fetus will have inherited one copy of the mutant gene from the mother and the other from the father, especially in those instances when the mother and father have the same mutation.

However, the small number of circulating fetal cells, amounting to 1-6 cells/ml of maternal blood,[49,51,52] presents a significant challenge to their detection. Their rare occurrence has called for the development of enrichment procedures and more sophisticated methods of identification. Numerous protocols have been developed, but, in general, fetal cell

isolation involves identification via fetus-specific markers followed by their capture from the maternal blood and confirmation of their fetal identity using additional markers. Only then can genetic analysis be performed on the identified fetal cells. This has been done primarily with two techniques: FISH using chromosome-specific probes and PCR to amplify unique fetal gene sequences.

Almost all significant fetal aneuploidies have been detected in fetal cells from the maternal blood.[53-57] These cases include all of the autosomal trisomies, some of the sex chromosome abnormalities, and triploidy. The development of PCR opened a whole new area of research because the low number of fetal cells present in a sample of maternal blood was no longer a limiting factor. Initially, most groups performed PCR with a Y chromosome-specific sequence to detect the presence of male fetal cells in maternal blood.[58-60] Subsequently, PCR was used to prove the presence of paternally-inherited fetal genes absent in the mother, e.g. β globin mutations, HLA DR and DQ alpha genes, and Rhesus D.[61-64] Still, one major concern regarding the use of fetal cells in maternal blood is that these cells can persist in the maternal circulation for years after delivery. Therefore, they can interfere with the prenatal diagnostics of a following pregnancy[14,65] and other sources of fetal DNA for prenatal diagnostics are being investigated.

Currently, the most promising source is cell-free fetal DNA. This new area of research developed following the discovery of large amounts of circulating cell-free tumor DNA in the plasma and serum of cancer patients.[66,67] Lo et al.[68] first demonstrated the presence of male fetal DNA sequences in maternal plasma and serum. Fetal DNA was detectable in as little as 10 ml of maternal plasma, accounting for 3.4% of the total cell-free plasma DNA in maternal plasma between 11 and 17 weeks of gestation.[69] Plasma samples obtained at term contained as much as 6.2% fetal DNA. Using the amplification of Y chromosome sequences as a detection method, none of the women pregnant with a female fetus and none of the nonpregnant control women had detectable fetal DNA levels.[69] Fetal DNA in maternal plasma can be detected as early as 5 weeks of gestation.[70,71] Whereas the maternal component of cell-free DNA is believed to be derived mainly from hematopoietic cells, the fetal material is believed to be derived from syncytiotrophoblasts in the form of apoptotic fragments.

Lo et al.[72] further investigated the clearance kinetics and turnover of fetal DNA in the maternal circulation. By examining plasma samples obtained from women during labor, immediately after delivery, and hours to days post partum, they showed that fetal DNA was cleared within 2 hours in most women. The mean half-life for circulating DNA was 16.3 minutes, suggesting that large quantities of fetal DNA 'enter' the maternal circulation continuously to sustain a steady state.

After its initial discovery, fetal DNA analysis in maternal plasma was soon shown to be feasible for the prenatal assessment of a number of fetal genetic traits. Applications have been reported for the assessment of fetal aneuploidy,[73,74] sex-linked disorders,[75] fetal RhD status,[76,77] congenital adrenal hyperplasia,[78,79] and β-thalassemia.[80] In particular, the prenatal prediction of fetal RhD status attained such high accuracy that its use has been introduced into the clinical setting.[76,81,82] In addition to the use of maternal plasma for the assessment of fetal genetic traits, the rapid clearance of fetal DNA has prompted investigators to study the potential use of fetal DNA quantification as a marker for fetomaternal well being. Elevated fetal DNA concentrations have been demonstrated in pregnancies associated with a variety of obstetrical complications, including pre-eclampsia. It appears that fetal DNA levels are not only elevated during pre-eclampsia,[83,84] the elevation predates the development of clinical symptoms,[84,85] and the degree of elevation corresponds with the severity of pre-eclampsia.[84,86] Other pregnancy-associated conditions linked with an elevated fetal DNA concentration include preterm labor,[87] fetomaternal hemorrhage,[88] invasive placentation,[89] hyperemis gravidarium,[90] and polyhydramnios.[91] Taken together, non-invasive prenatal diagnosis has become a true possibility, which is welcomed by women,[92] but much effort is needed to make it more widely applicable.

Phenotype of chimeric cells

Chimeric cells have been attributed to several phenotypes. The chimeric cells were first described as very peculiar cells - big in size with multiple nuclei. They were most likely syncytial trophoblast cells present in the lung capillaries of a woman who died of eclampsia.[4] Mueller et al.[93] later confirmed the trophoblast phenotype using antibodies against syncytiotrophoblast and cytotrophoblast cells. Trophoblast cells are not the only

kind of pregnancy-derived chimeric cells that have been found. Next to trophoblast cells, investigators tested pregnant women for chimeric nucleated red blood cells because they would undoubtedly be of fetal origin, and, indeed, these cells were present in the maternal circulation.[58] However, they were only present in the blood of pregnant women.

In 1969, Walknowska et al.[11] isolated Y chromosome-positive cells from peripheral blood sorted for leukocytes from healthy pregnant women. Some years later, using culture methods, chimeric leukocytes were defined more specifically as chimeric lymphocytes and granulocytes, with chimeric lymphocytes being present more frequently.[12,94] Only two decades ago, chimeric cell phenotyping began to be performed using PCR techniques and immunohistochemical staining. Using these methods, several studies confirmed that chimeric leukocytes could have various phenotypes, including T lymphocytes (CD3+),[14,95-99] further categorized into T helper cells (CD4+) and cytotoxic T cells (CD8+),[98,100] B lymphocytes (CD19+/CD20+),[96,99] monocytes/macrophages (CD14+),[96,99] and NK cells (CD56+/CD16+).[96,99]

Because umbilical cord blood contains a large number of progenitor cells, it was assumed that the fetus-derived chimeric leukocytes in the mother are differentiated from lymphoid progenitor cells. Bianchi et al.[14] investigated whether hematopoetic (CD34+) or lymphoid (CD34+CD38+) progenitor cells are present in peripheral blood of nonpregnant parous women and found that this was the case with hematopoetic progenitor cells being present more often than lymphoid cells, even in a woman that gave birth to a son 27 years earlier. The presence of chimeric hematopoetic progenitor cells (CD34+) was also confirmed by several other groups.[101,102] These findings are interesting as they may indicate that a continuous pool of chimeric progenitor or stem cells exists. The presence of chimeric cells in bone marrow sections from parous women underlines this hypothesis even more.[103] Only during the last decade have chimeric cells in tissues been investigated more extensively, demonstrating the presence of chimeric hepatocytes, epithelial cells, and cardiomyocytes.[104-107] These findings led to the attribution that chimeric cells have the capacity to proliferate and differentiate. However, for this capacity to exist a pool of mesenchymal stem cells would have to be present and only recently has some evidence been found.[103,108]

Routes of chimeric cells during pregnancy

In summary, during pregnancy there are three routes by which chimerism can be achieved. The first route is that chimeric fetal cells enter the maternal circulation via the placenta.[53,68] The second route is that chimeric maternal cells enter the fetus during pregnancy. It has been demonstrated that umbilical cord blood contains low numbers of maternal cells.[109] The third route is, in the case of a pregnancy with two or more fetuses, cells from one fetus enter the other fetus, and the other way around, leading to twin chimerism.[8,110,111] Of these three routes (fetal-maternal, maternal-fetal, and twin-twin route), it has been demonstrated that chimerism can persist after the completion of a pregnancy.[8,14,112]

There is a fourth possible route, for which no evidence has been published thus far, but which may be plausible. Namely, in the case of a pregnancy, cells from a previous pregnancy may enter the new fetus via the placenta leading to non-twin sibling-derived chimerism. Moreover, in theory, all kinds of chimeric cells that may be present in the mother can enter the fetus (i.e., also cells from the mother's mother or from a previous pregnancy).

Blood transfusion

Next to pregnancy, another potential source for chimeric cells is the transfusion of blood products. In 1999, Lee et al.[113] were the first to extensively study the survival kinetics of specific donor leukocyte (white blood cell, WBC) subsets in immunocompetent recipients after blood transfusion. Blood samples were collected from eight female patients who underwent elective surgery prior to transfusion and on days 1, 3, 5, 7, and 14 post-transfusion, and from a second group of 10 female trauma patients up to 1.5 years after transfusion. The WBC subsets from frozen whole blood were isolated using CD4, CD8 (T cell), CD15 (myeloid), and CD19 (B cell) antibody-coated magnetic beads. Donor WBCs were counted by quantitative PCR for the male-specific sex determining region (SRY) sequence. In all eight elective surgery patients who had received 1 or 2 units of non-leukodepleted male red blood cells (RBCs), 99.9% of male donor leukocytes had been cleared at 24 hours after transfusion. Unexpectedly, in six out of eight recipients, a substantial increase in Y chromosome-positive donor leukocytes was observed in

samples taken 3 or 4 days after transfusion. No donor leukocytes were detected in the recipients' circulation at 7 to 14 days post-transfusion in any of these women. This was in line with previously reported clearance kinetics.[114,115] Surprisingly, in the trauma patients transfused with 3-14 units of male RBCs, seven out of 10 exhibited a long-term persistence of donor-derived cells.[113] Two patients with the longest follow-up (18 months) showed donor cell survival up to this last time-point. Five patients showed donor cell survival up to 6 months; one of these became negative for male donor cells by 1 year follow-up, whereas the other four were positive at the last sampling time point. Between 0.5 and 10% of circulating WBCs in these recipients were donor-derived and involved multiple lineages (CD4, CD8, CD15, and CD19) of donor leukocytes. The samples collected from the two remaining patients became negative for donor cells between 4 and 6 months after transfusion. Even more intriguing were results from mixed leukocyte reaction (MLR) assays. The MLR assay assesses the response of T lymphocytes to a specific antigen challenge and compares two populations of cells that are mixed together after one of the populations has been inactivated by prior treatment with the antimetabolite mitomycin C. The assay measures the proliferation of the other population of lymphocytes in response to the alloantigenic profile of these inactivated cells. In the study by Lee et al. described above,[113] MLR assays were performed on blood samples from two patients showing a long-term persistence of donor-derived cells. Cells from both patients had a very low in vitro response when incubated with the cells of one specific donor compared to a normal response to the cells of the other donors (Figure 5). The cells from that specific donor responded against the recipient at a level comparable with those of the donors whose cells did not engraft. Interestingly, HLA DR and DQ testing of the cells surviving in the recipient's circulation, and the blood samples of all blood donors, confirmed that the surviving cells were most likely from that one single donor, as was demonstrated by the MLR assay.[113] These MLR assay and HLA testing results were confirmed in other studies.[116-118]

In light of these findings, it is not surprising that no direct relation has been observed in many studies between the number of units that are transfused and the persistence of donor cells in the recipient's circulation,[113,114,116-118] because cell survival depends more on the degree of HLA-matching than quantity. To further investigate the long-term

occurrence of donor cells in the recipient, Lee et al.[116] studied 27 trauma patients that received at least 2 units of RBCs. Five of them showed a long-term persistence of donor cells (median follow-up 26 months) with 0.40 – 4.90% of all peripheral WBCs being chimeric. The patient with the highest number of chimeric cells had received just 4 units of RBCs, so even small numbers of transfused units may be responsible for considerable long-term chimerism. Also in this study, the persisting donor cells appeared to be attributable to a single donor.

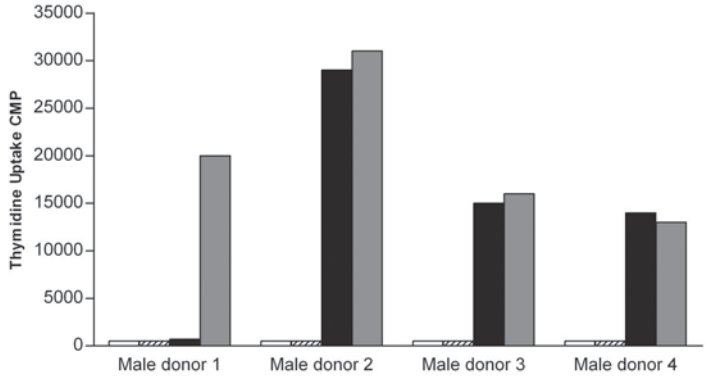

Figure 5. Example of mixed lymphocyte reaction (MLR) results from a single blood transfusion recipient that showed long-term chimerism and the donors of the four transfused units. White bars represent the tritiated thymidine uptake (a measure of proliferation) of cells from the recipient in response to inactivated (i.e. treated with mytomycin C) autologous cells (control). Patterned bars represent the thymidine uptake of the indicated donor cells in response to their own inactivated cells (control). Black bars represent the thymidine uptake of the recipient's cells in response to inactivated cells from the indicated donor. Grey bars represent the thymidine uptake of the indicated donor in response to inactivated cells from the recipient. Cells from the recipient show a very low response when incubated with the cells of donor 1 compared to the response to donors 2, 3, or 4. (Based on Lee et al.[113])

Several groups studied whether the transfusion of leukoreduced blood components decreased the likelihood of developing long-term chimerism as the concentration of donor WBCs is decreased 1000-fold (leukoreduced units contain approximately 10^6 WBCs per liter compared to 10^9 WBCs per liter for a non-leukoreduced unit). Interestingly, no decrease in persisting donor-derived cells was observed.[116,118]

Taken together, it seems that robust, long-term WBC chimerism has thus far appeared to be unique to patients being resuscitated for severe traumatic injury.[119] In contrast, patients receiving multiple transfusions for other conditions, such as human immunodeficiency virus infection,[120] hemoglobinopathies,[121] and elective orthopedic surgery,[113] have shown evidence of transient expansion followed by a fairly rapid and complete clearance of donor WBCs, but no long-term chimerism. Apparently, patient characteristics are also important for long-term chimerism. The large majority of trauma patients with transfusion-derived chimerism exhibited evidence of only one or two minor-type HLA alleles, suggesting that transfusion associated chimerism commonly involves only one donor despite some patients receiving blood products from a multitude of donors.

Transplantation

Next to pregnancy and blood transfusion transplantation, solid organ or bone marrow transplantation is a source for chimeric cells. After transplantation recipients are, per definition, chimeric because both situations lead to an individual containing tissues of diverse genetic constitution. Nevertheless, the term chimerism can be confusing, especially in these situations, because of its various forms of appearance. First, there is the transplantation of bone marrow or solid organ from one individual into another individual; this makes the recipient chimeric. Second, in both solid organ and bone marrow transplantation, donor-derived peripheral cells are present in the recipient's circulation; this also makes the recipient chimeric, though in a different way. Third, recipient-derived cells replace the donor organ's epithelial or endothelial cells; this makes the graft chimeric. Implications of chimerism in transplantation will be discussed in paragraph 5 of this chapter.

3. Possible implications of chimerism

The presence of chimerism in humans may have several implications. Firstly, the chimeric cells may be present in the host without interacting with the host's immune system. The possibility of this innocent bystander role of chimeric cells is supported by the fact that chimeric cells are frequently present in the blood of healthy individuals.

Secondly, chimeric cells may play a role in transplantation pathology. An individual who receives an organ graft is, per definition, a chimera, but there is also chimerism of the graft itself, which may enhance graft tolerance. It has been suggested that the replacement of donor cells by recipient cells in the graft makes the graft more 'self' resulting in improved graft tolerance. On the other hand, donor cells that enter the peripheral circulation of the recipient may influence the peripheral tolerance of the donor to cells of the graft.

Thirdly, chimeric cells may play a role in the initiation of autoimmune disease. Since it is known that chimerism may influence the immune system, it could be that, under certain circumstances, this process is disturbed and an immune response induced by chimeric cells may deteriorate into a loss of tolerance to self-antigens. This harmful role of chimeric cells is supported by observations that chimerism is present more often in individuals with autoimmune diseases (e.g. systemic sclerosis) than in healthy individuals. The three scenarios introduced above will be discussed in detail below.

4. Chimerism in healthy individuals

The occurrence of chimeric cells in the maternal circulation during and after pregnancy has been widely investigated. Chimeric cells can be identified in the circulation of almost all pregnant women.[49,52,70] These cells can be detected at as early as 5 weeks of gestation[71,122] and their numbers increase with advancing gestational age.[49,70,123] Even in women without clinical pregnancies, chimeric cells have been repeatedly found in blood and tissue specimens.[98,124,125] Most of these cells are most likely derived from unrecognized pregnancies. The termination of a pregnancy seems to be the particular event at which fetal cells enter the circulation,[123] with an observed difference between spontaneous terminations (i.e. spontaneous abortion or delivery) and induced abortions.[125,126] Chimerism seems to be significantly more frequent, and a higher number of chimeric cells are observed, in women with an induced abortion.

Also, in murine pregnancies, fetal cells can be detected in a variety of tissues including blood, spleen, liver, kidney, heart, lung, brain, and bone marrow.[127,128] The cells persist after delivery, but the time since the last delivery and the number of pregnancies are important parameters in the persistence of fetal cells. Fetal cells could be detected for up to the first 2 weeks after a first delivery. After three pregnancies, mice still had detectable fetal cells 3 weeks after delivery.[127] Histocompatibility between the mother and the fetus is an important influence on the number of fetal cells during murine pregnancy: female mice with an H-2^b genotype carrying congenic fetuses (i.e. also with an H-2^b genotype) have significantly higher numbers of chimeric fetal cells than mice carrying allogenic fetuses (i.e. mice with a H-2^b genotype carrying H-$2^{b/d}$ offspring).[127,129]

Since the first description that fetal cells can persist in maternal blood for up to 27 years,[14] much effort has been put towards investigating the long-term occurrence of chimeric cells.[65] Recently, O'Donoghue and colleagues[103] studied bone marrow and rib sections from nine women who had given birth to at least one son 13 to 51 years earlier, one woman who had never been pregnant, and four women with only daughters. They found male cells (as detected by XY FISH) in all nine samples from women with sons and in none of the five control women. The results were validated by PCR with three different Y chromosome-specific probes. In all samples, some chimeric cells were identified as mesenchymal stem cells because of their morphology and immunophenotype, self-renewal in vitro, and their ability for osteogenic and adipogenic differentiation. Intriguingly, these putative fetal mesenchymal stem cells were detected in women who had been pregnant with their youngest son up to 51 years earlier (median 36 years). These findings imply that fetal cells transferred during pregnancy engraft marrow and bone, where they persist for decades. The authors even suggested that "fetal stem cells in maternal marrow could also act as a long-term reservoir of stem cells and might even explain why women live longer than men."[103]

Studies like the one by O'Donoghue and colleagues have given rise to what is often called the 'repair hypothesis'; namely, during pregnancy, mothers acquire a population of fetal progenitor cells that can be recruited to maternal sites of injury and adopt the maternal tissue phenotype.[130] In animal models this has been extensively studied.[131-

[135] After giving birth to enhanced green fluorescent protein (EGFP)-transgenic fetuses, female rats were exposed simultaneously to ethanol and gentamicin to induce chronic liver and acute tubular renal injury, respectively. After this exposure, fetal hepatocytes could be detected in the maternal, damaged liver and, similarly, tubular cells of fetal origin were found in the maternal, damaged kidney.[135] Khosrotehrani et al.[133] investigated the role of fetal cells after specific murine hepatic injuries. After giving birth to EGFP-transgenic fetuses, either chemical or surgical liver injury was induced in the female mice by injecting carbon tetrachloride or by performing partial hepatectomy, respectively. The PCR results showed that, in chemical but not surgical injury fetal GFP-positive cells were detectable in the maternal liver and that the fetal cell presence was significantly increased over time following injury (4 weeks versus 8 weeks). These results suggest that specific types of injury may elicit different fetal cell responses in maternal organs.

Studies of chimerism in human tissue are scarce, but Srivatsa et al.[124] reported the presence of male fetal cells using FISH on the thyroids of women with various diseases and a history of a prior male child. In one case, fetal cells were found to be organized as multiple thyroid follicles indistinguishable from the adjacent maternal thyroid tissue and underlining the capability of fetus-derived chimeric cells to differentiate into organ-specific structures. In a following study, a high number of male cells were found in the liver biopsy of a woman affected with chronic Hepatitis C.[136] DNA polymorphism analysis indicated that the probable source of the male cells in her liver was a pregnancy the woman had terminated almost two decades earlier. The male cells in the liver were morphologically identical to the surrounding liver tissue, which suggests that they were hepatocytes.[136] Therefore, it seems that, at least in some cases, fetal cells may play a role in maternal repair.

5. Chimerism in transplantation

In the early 1960s, Medawar hypothesized on the replacement of donor endothelium by recipient endothelium in the kidney.[137] He suggested that, in time, the endothelium

of donor origin would become 'owned' by the recipient, which would lead to graft adaptation, a condition first described by Woodruff in 1950.[138] Medawar believed that graft adaptation could be induced by the focal replacement of graft endothelial cells by recipient cells, reducing the foreign antigen load. The most likely reason for endothelial replacement in a transplanted organ would be a loss of the original endothelial cells due to, for instance, acute rejection, ischemia, or medication toxicity. Endothelial progenitor cells replace the damaged endothelium in the kidney after injury, a phenomenon that has been studied extensively in animal models.[139] Evidence that a similar process takes place in humans was obtained by Lagaaij et al.,[140] who tested 38 kidney allografts for the presence of recipient-derived endothelial cells. Chimeric cells were found significantly more often in the grafts of patients who experienced rejection episodes and chimerism appeared to be more extensive after vascular rejection than after interstitial rejection.

Still, the exact link between the occurrence of chimerism in allografts and rejection is unclear. As suggested previously, it is possible that the induction of chimerism by the replacement of donor cells with cells of recipient origin has a favorable role as it may reduce alloreactivity. On the other hand, it may be that injury caused, for instance, by vascular rejection can induce chimerism in the graft because damaged endothelial cells may be replaced by recipient progenitor cells. Once chimerism is present, this may have no further role, or it may reduce alloreactivity and make the organ less prone to further rejection episodes, thereby improving graft survival.

Not only can the endothelium of a renal graft be replaced by cells of recipient origin, but tubular epithelial cells in a renal graft may also become chimeric.[141] This finding corresponds to the results of other studies on chimerism in transplanted organs: chimeric cardiomyocytes and smooth muscle cells have been found in transplanted hearts;[142-146] chimeric endothelium, duct epithelium, and hepatocytes have been found in transplanted livers;[147-149] and chimeric bronchial epithelium, endothelium, and type II pneumocytes have been found in transplanted lungs.[150,151] Differences have been observed in the reported numbers of chimeric cells in allografts, e.g. in transplanted hearts ranging from none to 9% chimeric myocytes.[142,143,145] These different results may be due, in part, to the different techniques and protocols used to detect the chimeric

cells. These will be more elaborately discussed in chapter 4.
Interestingly, there seems to be a gender difference in the occurrence of chimerism, though studies investigating these subjects are scarce. Van Poelgeest et al.[152] studied 85 renal transplant biopsies of 24 patients and found endothelial chimerism in 27 of the 85 biopsies from 16 of 24 patients. All eight female patients, but only half of the sixteen male recipients, had endothelial chimerism in their grafts. Also, in female recipients, this occurred significantly earlier than in the male recipients. In light of these findings, it is interesting that the long-term outcome of donor-kidney-recipients has been described in large studies to be better in female than male recipients, both in humans and animals.[153,154] This difference has also been described in liver transplantation.[155] It is currently unknown which factors could contribute to better graft survival in women, but it could be possible that the higher occurrence of chimerism in the transplanted allograft plays a role.

Obviously, other factors than chimerism are likely to have a major influence on graft survival, such as HLA compatibility, cold ischemia time, age of the donor, the level of reactive HLA antibodies before transplantation, plus the age and race of the recipient.[156,157] Graft survival is optimal when donor and recipient are HLA identical,[157] as is the case with an HLA-identical sibling. However, in most situations, the donor and recipient are only partially HLA-identical with siblings, parents, offspring, and spouses serving as donors. The concept that exposing the immune system of the child to noninherited maternal antigens (NIMA) during pregnancy and breast feeding might have lifelong consequences for tolerance was first formulated in the 1950s[158] (Figure 6). The NIMA effect was forgotten during the following decades until recent years. In 1998, Burlingham et al.[159] retrospectively studied graft survival in 205 patients who had received renal transplants between 1966 and 1996. They found that graft survival was significantly higher in recipients of kidneys from siblings expressing maternal HLA antigens not inherited by the recipient than in recipients of kidneys from siblings expressing paternal HLA antigens not inherited by the recipient (10-year graft survival of 77% and 49%, respectively). Interestingly, there was a higher incidence of early rejection in the former group. Other studies showed better graft survival when NIMA haplo-identical siblings were used as bone marrow donors. Van Rood et al.[160] observed

significantly less graft-versus-host disease and increased patient survival when NIMA haplo-identical siblings were used as donors. Furthermore, Japanese transplant centers have successfully transplanted NIMA haplotype-mismatched sibling and maternal stem cells into patients without T cell depletion.[161,162] Patients and donors included in their reports were all chimeric for the mismatched haplotype. Chimerism during pregnancy may be an important factor for the induction of NIMA-specific tolerance.

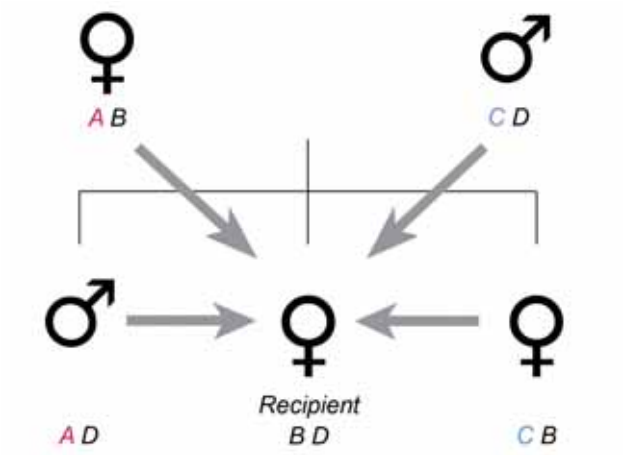

Figure 6. Hypothetical case of inherited and noninherited maternal and paternal HLA alleles in a female transplant recipient with end-stage renal disease. Arrows indicate the potential sources of kidney transplants from living related donors (two parents and two siblings); all are haplo-identical to the recipient. All potential donors have HLA antigens inherited by the recipient, but they also express either maternal antigens not inherited by the recipient (red) or paternal antigens not inherited by the recipient (blue). From Burlingham et al.,[159] with permission.

In solid organ and bone marrow transplantation, donor-derived peripheral cells spread through the recipient's circulation and organs. Thomas Starzl and colleagues were the first to propose, in the early nineties, that the exchange of migratory leukocytes between the transplant and the recipient, with consequently long-term cellular chimerism in both, is the basis for acceptance of whole-organ allografts.[163] In several patients with kidney or liver transplants it has been possible to decrease or completely stop immunosuppressive therapy.[164,165] Indeed, donor-derived cells have been extensively found in the blood, lymph nodes, and skin biopsy specimens of recipients of different organs.[163,165] However, taken together, the data on recipient chimerism, transplantation, and rejection is inconclusive. In one meta-analysis, chimerism in solid

organ transplantation was associated with a higher incidence of acute rejection for heart, lung, and kidney transplants, and alternatively with a lower incidence in liver transplants.[166] However, other studies have shown that the presence of chimerism is not related to graft tolerance, rejection, or clinical outcome.[167]

6. Chimerism in autoimmunity

In 1996, Lee Nelson suggested for the first time that chimerism may be involved in the pathogenesis of autoimmune diseases.[168] She based this hypothesis on three observations: 1) the persistence of fetal chimerism in the mother, 2) the strong predilection of the autoimmune disease, systemic sclerosis (SSc), for women with a peak incidence following child bearing years, and 3) the clinical similarities of systemic sclerosis to chronic graft-versus-host disease that occurs after allogenic bone marrow transplantation.[169] The hypothesis stated that, similar to the response seen after allogenic bone marrow transplantation, autoimmune diseases are the result of foreign (i.e. chimeric) cells reacting against host tissues, implicating that some autoimmune diseases are actually alloimmune diseases. Nelson started investigating the presence of chimerism in the blood of women with systemic sclerosis using PCR.[170] In 10 of the 17 women with SSc that were investigated, male DNA was found, compared to 4 of the 16 healthy controls. The mean number of male cell DNA equivalents per 16 ml of blood from the patients was 11.1, and 0.38 for controls. These findings demonstrated that low concentrations of male DNA can be detected both in women with SSc and healthy controls, but the occurrence and amount of male DNA is significantly higher in patients with SSc. Other studies found similar differences in the presence of chimerism between patients with SSc and healthy controls;[95,171-174] however, there was not always a significant difference between the patients and controls.[96,98,175-177] The presence of chimerism in healthy controls underlines that the presence of chimerism alone is not enough to induce an autoimmune response. Therefore, other factors, for example the phenotype of the chimeric cells or HLA similarities between the chimeric cells and the host cells, may be important as well.

In the scenario that a graft-versus-host-like response would be responsible for the

induction of disease, the chimeric cells would have to be immunological cells, especially T cells, and they would be present predominantly at sites of injury. Artlett et al.[95] investigated the phenotype of chimeric cells extracted from the blood and skin lesions of women with SSc and found that chimeric T cells and monocytes were present in both peripheral blood and skin tissue derived from the lesion sites. The T cell group consisted of both chimeric T helper cells and chimeric cytotoxic cells.[100] Scarletti et al.[178] investigated whether chimeric T cells present in patients with SSc react against the cells of the host. They isolated T cell clones from women with SSc that showed a proliferative reaction to antigens on irradiated non-T cells from the same women and investigated whether these clones were male (i.e. chimeric). Of all the responsive clones isolated from women with SSc, 7 of 39 were chimeric in contrast to 1 of 11 clones isolated from healthy controls. The immunologic capacity of the reactive clones was confirmed by demonstrating cytokine production in reaction to the host cells. The existence of a competent immune response between chimeric T cells and cells from the host was further underlined by the demonstration that the proliferative response could be blocked completely in vitro by adding anti-MHC class II antibodies, thereby preventing the binding of chimeric T cells to host cells. With this study, all of the requirements for Nelson's theory were met.

In other autoimmune diseases, such as Hashimoto's thyroiditis, Sjögren's syndrome, and primary biliary cirrhosis, scientists have investigated the presence of chimerism, which led to different results.[107,124,179-186] However, in none of these diseases is the number of published articles or the evidence in favor of Nelson's theory as high as in systemic sclerosis. The presence of chimerism has not only been investigated in adults with autoimmune diseases, but also in children, both healthy and with immune-mediated diseases. In immunocompetent healthy infants, maternal cells have been found in 20-100% of umbilical cord blood samples.[187-190] Moreover, it has been demonstrated that these cells are not necessarily eliminated by the child's immune system but can persist into adult life.[99,112] The question arises of whether chimeric cells can elicit an autoimmune response in children in the same way as has been suggested in adults. Firstly, chimeric cells have been found in children with immune-mediated disease; for example, in the hearts of children that had developed lupus neonatal heartblock.[191] Also,

chimeric cells have been found in the muscle biopsies of infants with idiopathic myositis, skin biopsies of infants with pityriasis lichenoidides, and skin biopsies of infants with dermatomyositis.[192-194] Secondly, Loubiere et al.[99] demonstrated that maternally-derived chimeric cells can have immunogenetic phenotypes (T lymphocytes, B lymphocytes, monocyte/macrophages, and NK cells), indicating that an immune response in children from maternal cells is possible.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease that was first described in the 19th century. It is a chronic, relapsing, inflammatory disorder of the connective tissue that can affect almost every organ system. Symptoms vary from person to person and may come and go resulting in periods of relative quiescence and periods of exacerbations. The disease is characterized by immunological abnormalities and demonstrated by the presence of autoantibodies, in particular antinuclear antibodies (antinuclear factor and anti-double-stranded-DNA antibodies), anticytoplasmic antibodies, and antiphospholipid antibodies. Because symptoms differ per patient, the American College of Rheumatology has formulated diagnostic criteria (Table 1)[195,196] comprising characteristic abnormalities of the skin, joints, serosal membranes, kidneys, neurological system, hematological system, and immunological system. A person is diagnosed with SLE if four or more of 11 criteria are present, serially or simultaneously. Together with these classified, relatively characteristic symptoms, non-specific symptoms also often occur, including fatigue, fever, weight loss, and Raynaud phenomenon.

Renal involvement in the form of lupus nephritis is a serious event. Up to 60% of patients develop renal involvement during the course of their disease, sometimes even in the absence of abnormal urinary or serum parameters.[197] The anatomy and physiology of the kidney makes it highly susceptible to inflammatory insults caused by autoantibodies.[197] Lupus nephritis remains a major cause of renal failure and mortality among patients with SLE.[198] The World Health Organization has developed classification criteria to categorize the different pathological forms of lupus nephritis. This classification has recently been updated by the Renal Pathology Society and the International Society of Nephrology.[199]

Table 1. Criteria for Classification of Systemic Lupus Erythematosus (SLE).[195, 196]

Malar rash
Discoid rash
Photosensitivity
Oral ulcers
Arthritis
Proteinuria (>0.5 gr/24u) or cellular casts
Seizures or psychosis
Pleuritis or pericarditis
Hemolytic anemia, leukopenia, lymphopenia, or thrombocytophenia
Antibody to DNA or Sm antigen, or phospholipids
Positive immunofluorescent antinuclear antibody

The diagnosis of SLE requires the presence of 4 of the 11 criteria, serially or simultaneously, during any interval of observation.

The prevalence of SLE is about 1:2,000 with African-Americans, Hispanics, and Asians being affected more often than Caucasians,[200,201] and SLE occurs more commonly in women than in men (overall female: male ratio is 9:1), but the magnitude of this difference differs per age group (Figure 7).[202] The first symptoms can occur from early childhood up to 85+ years with a peak incidence between the ages of 29 and 54.[202]

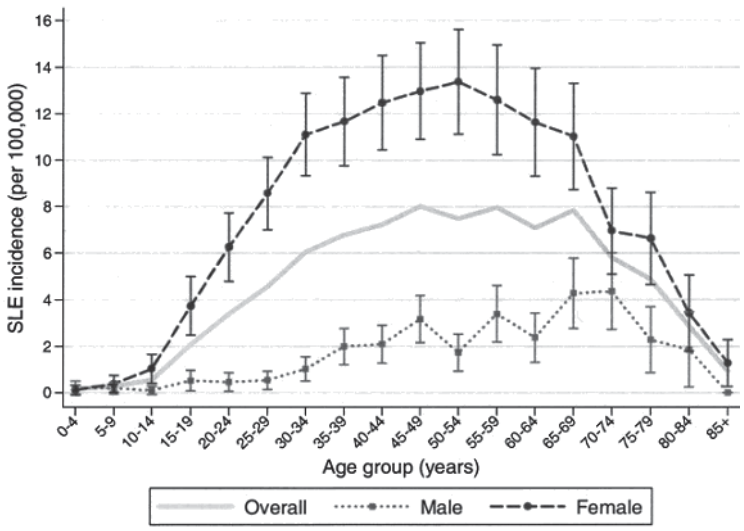

Figure 7. Age- and sex-specific incidence rates of systemic lupus erythematosus (SLE; per 100,000) with 95% confidence intervals. From Somers et al.,[202] with permission.

The etiology of SLE is unknown, but it is likely that several factors are involved.[203-205] First, hormonal factors may play an important role; because 90% of SLE patients are females, a disease-inducing role for female hormones and/or a protective role for male hormones seems likely.[204,205] However, it is unclear how sex hormones could promote SLE and trials with sex hormone treatments have had disappointing effects. Second, infectious agents may induce specific immune responses by molecular mimicry leading to SLE. In relation to this scenario, a role of the Epstein-Barr virus (EBV) has been described and EBV DNA has been found to be present in 99% of SLE patients, in contrast to 70% in controls.[206] However, a causal relationship between EBV and SLE has not been demonstrated. Thirdly, genetic susceptibility may play a role in the development of SLE. Monozygotic twins have a concordance rate of 25% for SLE, and dizygotic twins have a rate of 2%.[207] These rates indicate that a genetic contribution is important, but it is not sufficient to cause the disease by itself. Fourthly, certain environmental triggers have been associated with SLE, of which, ultraviolet radiation is the most important.[204] However, these environmental triggers alone are not sufficient to cause SLE. It is more likely that they can induce an immune response in a susceptible patient, which may lead to SLE under certain circumstances. Fifthly, several immunopathological factors have been demonstrated that could possibly play a role in the development of SLE;[204,205] for instance, the hyperactivation of B cells, hyperactivation of T cells, abnormal phagocytic function, and abnormal immunoregulation. These immunological abnormalities would, amongst others, lead to defective apoptosis and result in an exposure to self-antigens that are normally degraded within the cells without exposure, which may eventually trigger an immune response leading to the production of auto-antibodies and subsequent tissue injury.

Chimerism and systemic lupus erythematosus

In line with Nelson's theory that chimerism may be involved in the pathogenesis of SSc, chimerism may also play a role in the pathogenesis of SLE. There are several arguments in favor of this theory, which will be outlined in detail in chapter 7 of this thesis. Looking at the three arguments on which Nelson's theory is based, 1) the persistence of fetal chimerism in the mother, 2) the strong predilection of systemic sclerosis in women with a peak incidence following childbearing years, and 3) the clinical similarities of systemic sclerosis to chronic graft-versus-host disease that occurs after allogenic bone marrow

transplantation,[169] the question of whether these arguments can also be applied to SLE arises. The persistence of chimerism as a result of pregnancy, or other sources, has been recognized in SLE and was described above. Furthermore, chimerism has been found in the peripheral blood of women with SLE and in a small number of organs.[208-211] The second argument, namely the peak incidence following childbearing years, is particularly true for SLE (Figure 7). Since fertile women have a relatively high chance of being chimeric as a result of (unrecognized) pregnancies, the peak incidence of SLE during or following childbearing years makes a role for chimerism in the pathogenesis of SLE more likely. However, a strong relationship between pregnancy and the occurrence of SLE has never been described. Nevertheless, a study by Grimes et al.[212] studied reproductive factors in women with SLE before and at the time of diagnosis and found that a prior hysterectomy or tubal sterilization had a protective effect. The possible relationship between pregnancy and the presence of chimerism has not been previously investigated in patients with SLE, and this is the topic of investigation in chapter 9 of this thesis.

Nelson's third argument regarding the clinical similarities between SSc and chronic graft-versus-host disease (cGvHD) cannot be applied to SLE as easily as for SSc. Although both cGvHD and SLE are systemic disorders affecting many organs and resulting in systemic symptoms like malaise and fatigue, there are many clinical and pathological differences between the two diseases. For example, the kidney is often affected in SLE, whereas, in cGvHD, renal involvement is rarely present. Instead, the intestines, skin, and serosa are affected.

In mice, an experimental model of graft-versus-host disease was developed in the early eighties, in which, F1 hybrid mice were injected with parental T cells.[213] The response of the F1 mice differed according to the genetic makeup of the donor and host. In most cases, the injection of parental T cells resulted in the development of a lethal graft-versus-host disease in the recipient. However, under certain circumstances, a condition resembling human SLE ensued accompanied by a proliferative glomerulonephritis with deposition of immunoglobulins and complement, characteristic of lupus nephritis.[213] This finding demonstrates that, despite the limited clinical similarities, a graft-versus-host response may lie at the basis of the development of SLE.

7. Outline of this thesis

The research described in this thesis is comprised of three major topics. First, the occurrence of chimerism in normal organs is described. In **Chapter 2** we report on the presence of chimerism in the kidneys, liver, heart, and spleen of women with sons and women without children and discuss the possible influence of chimerism in these organs on transplantation studies. In addition, the presence of chimerism in the lungs, skin, thyroid, and lymph nodes of women with sons are investigated in **Chapter 3**, and the possible implications of these findings for autoimmune diseases are discussed.

Secondly, we focus on the role of chimerism as a result of transplantation. Many papers have been published investigating if the amount of chimerism in the graft is related to transplantation outcome and the different methods used to investigate chimerism. A detailed review on this subject is given in **Chapter 4**. It is known that donor-derived cells can be detected in the recipient's circulation and peripheral tissues after solid organ transplantation. In **Chapter 5**, we investigate whether donor-derived cells are present in the skin tumors of patients with a renal allograft.

Thirdly, the role of chimerism in SLE is discussed. In **Chapter 6**, the presence of chimerism in kidneys with lupus nephritis is demonstrated and it is investigated whether chimeric T cells are present. The increased presence of chimerism in lupus nephritis led to the question of what the role of chimerism could be in the pathogenesis of SLE. This question is addressed in **Chapter 7** in which an outline of the literature with respect to three different hypotheses on the role of chimerism in SLE is given. The occurrence of chimerism in organs other than the kidney is investigated in **Chapter 8**. Whether a relationship exists between the presence of chimerism in kidneys of women with SLE and lupus nephritis, and their pregnancy history, is investigated in **Chapter 9**. **Chapter 10** reports on the presence of male-derived cells in female reproductive organs in women with SLE and healthy women. In **Chapter 11**, the role of chimerism in childhood lupus nephritis is addressed. The results of the studies described in this thesis are summarized and discussed in **Chapter 12** and our conclusions are postulated. **Chapter 13** provides a summary, general discussion, and conclusions in Dutch.

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