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Chimerism in health, transplantation and autoimmunity

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3

CHIMERISM OCCURS IN THYROID, LUNG, SKIN AND LYMPH NODES OF WOMEN WITH SONS

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ABSTRACT

Background

Chimerism indicates the presence of cells from one individual in another. Pregnancy and blood transfusions are considered the main sources for chimerism. Chimeric cells have been attributed a pathogenic role in various autoimmune diseases. However, data on the occurrence of chimeric cells in normal organs are scarce. In order to gain insight into the possible pathogenic potential of chimeric cells in autoimmune disease, it is necessary to determine the prevalence of chimeric cells in organs not affected by autoimmune disease.

Methods

In situ hybridization for the Y chromosome was performed on organs obtained at autopsy of 51 women. We investigated 44 thyroid, 38 lung, 21 skin, and 7 lymph node samples. All women had sons, and data from their blood transfusion histories were retrieved for at least 10 years before death. Slides were scored semi-quantitatively for chimerism as low (1-3 Y chromosome-positive cells per slide), moderate (4-10 positive cells per slide), or high (more than 10 positive cells per slide).

Results

Y chromosome-positive cells were found in 8 thyroid, 10 lung, 3 skin, and 1 lymph node samples, of 18 women. There was no association between the presence of chimeric cells and blood transfusion history. Most organs in which chimerism was present contained a small to moderate level.

Conclusion

Chimerism can occur in normal organs of women without autoimmune disease. Our results indicate that chimerism is not necessarily associated with disease.

INTRODUCTION

Chimerism indicates the presence of cells from one individual in another individual. These cells can either be circulating or they can be integrated into tissues. Lee Nelson was the first to hypothesize that chimeric cells may be involved in the pathogenesis of autoimmune diseases.¹ In recent years the role of chimeric cells in the pathogenesis of autoimmune diseases has been investigated. Most of these studies focused on chimeric cells in the circulation, by identifying non-self DNA in peripheral blood.^{2,3} A few studies examined whether chimeric cells were present in affected tissues, such as thyroid in Hashimoto's thyroiditis;^{4,5} and skin in systemic sclerosis^{6,7} and systemic lupus erythematosus.^{8,9} Although in some of the studies in which chimeric cells were identified in the peripheral circulation a difference was found between patients with immune-mediated diseases and healthy controls,^{10,11} in most of the studies the occurrence of chimerism was similar in patients and controls.¹²⁻¹⁴ In the studies in which the presence of chimeric cells in affected tissues was investigated, the control groups mostly consisted of patients with other diseases, not persons without disease, and the results from these studies vary extensively.^{4,15-17} Therefore, data on the occurrence of chimeric cells in normal tissues are essentially not available. Various hypotheses have been proposed on the role of chimeric cells in autoimmune diseases such as systemic sclerosis and systemic lupus erythematosus.^{18,19} Several studies investigated HLA relationships between the host and chimeric cell populations in autoimmune diseases, and observed increased bidirectional HLA class II compatibility.^{11,20} This could suggest that the higher number of chimeric cells present in autoimmune disease is not influencing the development of autoimmune disease, but instead, results from the immunogenetically similarities between the two cell populations. Another explanation may be that if host and chimeric cell populations look similar (i.e. are HLA Class II compatible) but are not identical (i.e. are different in Class I and minor antigens), an immune response targeting the chimeric cells might inadvertently target the "self" and trigger an autoimmune disease. This may explain how fetal cells can trigger an autoimmune disease in the mother. However, before any conclusions on the pathogenic potential of chimeric cells can be made, it is necessary to establish the distribution of chimeric cells in organs without disease.

Because most autoimmune diseases occur predominantly in women, identification of the Y chromosome is a useful method to study the presence of chimeric cells. It has been demonstrated that Y chromosome-positive cells in women are derived from previous pregnancies with male fetuses.²¹⁻²³ All women in the present study had sons, making it possible to identify the maximum number of Y chromosome-positive chimeric cells in the normal female population. Although they contain an extremely low proportion of nucleated cells, blood transfusions are an alternative source for chimerism.^{24,25} Therefore, of all the women in the present study, data from the blood transfusion history were retrieved for at least 10 years before death. Our results provide crucial data about the physiologic distribution of fetal chimerism, essential for future research on chimerism in autoimmune disease.

3

PATIENTS AND METHODS

Patients

Tissue specimens from thyroid, lung, skin, and lymph nodes were obtained from consecutive autopsies of women performed at the Leiden University Medical Center (LUMC) between 1999 and 2001. Clinical data containing the life-long histories of the patients were known from their medical records. Permission of the medical-ethical committee of the LUMC was obtained to enquire general practitioners about parity of the women. Patients with a history of autoimmune disease, solid organ transplantation, bone marrow transplantation, or stem cell transplantation were excluded. Eventually, tissue specimens of 51 women with sons entered the study.

Blood transfusion status

Data on blood transfusions were obtained from the Department of Immunohematology and Blood Transfusion of the LUMC. Data on the sexes of the blood donors were obtained from Sanquin National Dutch Blood bank. Data on blood transfusions were available from 1989 onwards. Beginning from 1995, also data on the sexes of the blood donors were available. Therefore, data on the sexes of the blood donors were not available in 12% of all transfusions, all from the period 1989-1994.

Histological Analyses

Tissues specimens were fixed in 4% neutral-buffered formalin and embedded in paraffin, according to hospital protocol. Paraffin-embedded specimens were stored in an air-conditioned room, with a constant temperature and air humidity. Tissue specimens of the thyroid, lung, skin, and lymph node samples were reviewed by light microscopy, and those specimens showing signs of autolysis or discrete lesions were excluded. Six lung specimens and two thyroid specimens were excluded; the lung specimens because of autolysis or because they contained intra-alveolar macrophages indicative of heart failure, and the thyroids because of autolysis. The remaining 110 tissue specimens were histomorphologically normal. To verify the quality of the tissue samples for the detection of sex chromosomes, in situ hybridization for the X chromosome was performed according to the same method as described below, using an X chromosome-specific DNA probe²⁶ on a random selection of various organ samples of 46 specimens. All specimens examined showed satisfactory X chromosome staining. Because only a selected number of organ specimens are obtained at autopsy, the number of available tissue specimens of each organ varied, and e.g. lymph node specimens were infrequently available. Area surface measurements were performed using digital image analysis with the program Image Tool (University of Texas, Health Science Center, San Antonio, Texas, USA) to compare the mean area of tissue evaluated of each organ.

In situ hybridization

In order to detect chimeric cells, in situ hybridization of the Y chromosome was performed as described earlier.²⁷ The sensitivity of the in situ hybridization was 58%, based on the findings in male control tissue, in which 58.3% of 1659 counted nuclei showed a positive signal for the Y chromosome. This level of sensitivity is comparable to that in other studies.²⁸⁻³⁰ By nested PCR and sequencing we confirmed that the probe was specific for the Y chromosome.²⁷ As a negative technical control, a male tissue sample was used on which the complete in situ hybridization protocol was performed, but instead of the hybridization mixture with the Y chromosome probe, only the hybridization mixture was added. This negative control was consistently negative.

Scoring

All slides were evaluated by two observers. Dots were only scored positive if they were present inside nuclei, if they had a similar size and staining intensity as those of the positive control samples, and if the background was completely clear. The degree of chimerism in positive samples was scored semi-quantitatively as low (1 to 3 Y chromosome-positive cells per slide), moderate (4 to 10 positive cells per slide), or high (more than 10 positive cells per slide). Area measurements were used to calculate the number of chimeric cells per mm² per organ, after the scoring was completed.

Statistics

Categorical variables were compared with the use of the chi-square test. Normally distributed continuous variables were compared with the Student's t-test, and non-normally distributed continuous variables with the Mann-Whitney U test. P values of < 0.05 were considered statistically significant. All statistical analyses were performed by SPSS 14.0.

3

RESULTS

Clinical data of the 51 women in the study are given in Table 1. In situ hybridization of the Y chromosome was performed on a total of 110 organs. Y chromosome-positive cells, as determined by in situ hybridization, were found in 8 of 44 thyroid (18%), 10 of 38 lung (26%), 3 of 21 skin (14%), and 1 of 7 lymph node samples (14%). In our study, there was no association between the presence of chimeric cells and age, or between the presence of chimeric cells and the cause of death.

In total, 18 of 51 women showed Y chromosome-positive cells in at least one organ. The age of these 18 women ranged from 47 to 81 years. Four women showed Y chromosome-positive cells in more than one organ (Table 2); three of them had never received a blood transfusion. Two of these four women showed Y chromosome-positive cells in lung and thyroid (patients 6 and 16), one in thyroid and lymph node tissue (patient 9), and one in lung and skin tissue (patient 10).

Table 1. Clinical characteristics

Number of women	51
Mean age (yrs)	63.0
Age range (yrs)	29 - 93
Cause of death:	
Infectious	11
Cancer	13
Cerebral	10
Vascular/myocardial	13
Other	4, i.e. liver cirrhosis from alcohol abuse (2x) amniotic fluid embolus cachexia
At least 1 blood transfusion from male donor	31

Table 2. Women with a Y chromosome identified by ISH in at least one organ

Patient	Age at death	Thyroid	Lung	Skin	Lymph node	Blood transfusion	Cause of death
1	47	++				Yes	Pneumonia
2	55	-	-	+		Yes	Carcinoma of the ovary
3	57	-	+			No	Neuro-endocrine carcinoma
4	57	-	+++			Yes	Multiple myeloma
5	62	-	-	++		Yes	Subdural hematoma
6	65	+	+	-		No	Neuro-endocrine carcinoma of the small bowel
7	67	-	+++			No	Myocardial infarction
8	67	-	+			Yes	Endocarditis
9	69	+		-	+	Yes	Respiratory/cardiac insufficiency
10	71	-	+	+	-	No	Pneumonia
11	74		+			Yes	Left ventricular fibrillation
12	76	+	-			Yes	Cardiac failure
13	77	++	-			Yes	Sepsis
14	77	-	+	-		Yes	Bronchopneumonia
15	81	++	-	-		No	Ruptured aneurysm of the vertebral artery
16	81	+	+			No	Myocardial infarction
17	81	+	-			Yes	Cardiac failure
18	81	-	+	-		No	Bronchopneumonia

ISH, in situ hybridization; -, no Y chromosome-positive cells; +, low: 1-3 Y chromosome-positive cells per slide; ++, moderate: 4-10 Y chromosome-positive cells per slide; + + +, high: >10 Y chromosome-positive cells per slide

The thyroid specimens had a mean surface area of $277 \pm 114 \text{ mm}^2$. The positive cells were mainly present in follicles (Figure 1A). Table 3 summarizes the finding of chimerism in relation to the cause of death and previous blood transfusions. The skin specimens had a mean surface area of $242 \pm 95 \text{ mm}^2$. The positive cells were present in the epidermis and seemed to be keratinocytes (Figure 1C). In the 7 lymph node specimens, low-level chimerism was found in one lymph node which had a surface area of 146 mm^2 . The chimeric cells seemed to be lymphocytes.

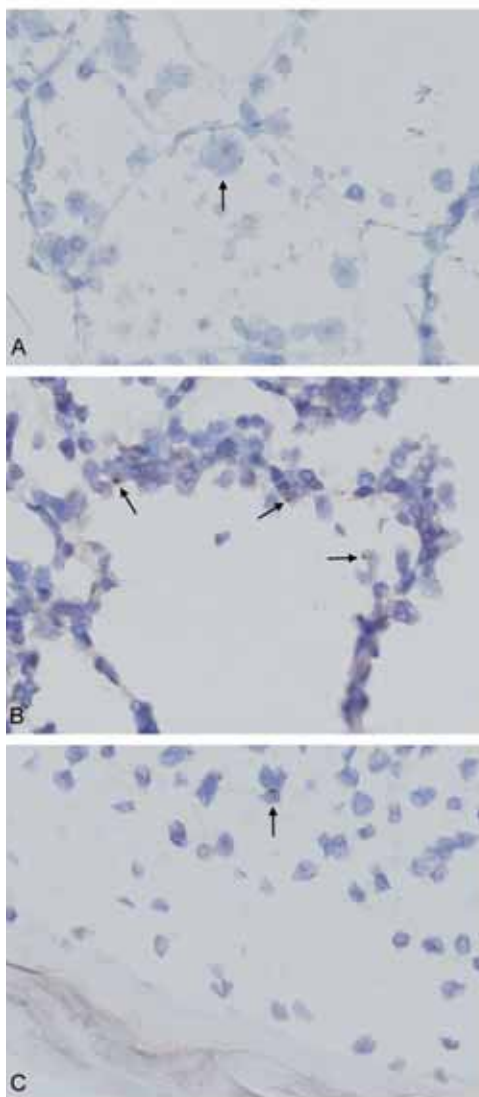


Figure 1. Red-brown dots indicating detection of the Y chromosome by in situ hybridization. (A) Thyroid: a positive cell lies in a follicle; (B) Lung: several positive cells are present in the alveolar wall; and (C) Skin: a positive cell is present in the epidermis.

The lung specimens had a mean surface area of $266 \pm 86 \text{ mm}^2$. The positive cells were mainly present in alveolar septae (Figure 1B). None were seen in bronchi or bronchioli. Sometimes, positive cells were found in alveolar spaces, raising the possibility that they were inflammatory cells. The lung was the only organ in which a high level of chimerism was found (Table 2: patients 4 and 7). In two lung specimens the chimeric cells were seen in clusters, i.e. at least 3 chimeric cells within one high power field of 400x (e.g. Figure 1B). In most lung and other organ specimens the chimeric cells were solitary cells (e.g. Figure 1A and C). A high degree of Y chromosome-positive cells was detected in lung specimens of two women. One of these women died of multiple myeloma, the other died of a myocardial infarction. One of the women in our study died of an amniotic fluid embolus, shortly after the delivery of a son. In her lungs, we found groups of cells that carried the Y chromosome in their nuclei. These groups of cells were morphologically recognizable as embolism. Therefore, they were not considered representative of the fetal chimerism as investigated in this study. At other sites in the lung of this woman, chimeric cells were not found. Other organs of this patient that we investigated (thyroid and skin) did not appear to have chimeric cells.

Table 3. Features of blood transfusion

Characteristic	No chimerism (N=20)	Chimerism (N=11)	p-value
Units of male PRBCs transfused	6.0 (2.5, 14.0)	5.0 (3.5, 9.5)	0.65
Time from last transfusion to death (days)	4.0 (2.0, 22.0)	4.0 (2.5, 276.0)	0.63
Mean time of transfusions to death (days)	12.6 (3.1, 225.3)	9.3 (3.9, 309.8)	0.97

Results are expressed as median and interquartile range. PRBCs, packed red blood cells

Thirty-two of 51 women received at least one unit of packed red blood cells (PRBCs), with a total of 428 units. Fifty-three percent, i.e. 225 units, were derived from a male blood donor. One of the 32 women only received PRBCs from a female donor; 31 women received at least one unit of male PRBCs. Blood transfusion features are given in Table 3. Of the 31 women with at least one male PRBC transfusion, 11 (35%) had Y chromosome-positive cells in one or more organs. Of 20 women without histories of blood transfusion, also 35%, namely 7 women, had Y chromosome-positive cells in one or more organs. No significant difference was observed in this study group in

the women with or without Y chromosome-positive cells with respect to the number of transfused units of male PRBCs, the time from the last transfusion to death, and the mean time of transfusions to death.

DISCUSSION

In this study we investigated the occurrence of chimeric cells in thyroid, lungs, skin, and lymph nodes from women who delivered male fetuses. Y chromosome-positive cells were identified in organs of 18 women, ranging in age from 47 to 81 years. Chimeric cells were present in 8 of 44 thyroid specimens, 10 of 38 lung specimens, 3 of 21 skin specimens, and 1 of 7 lymph nodes. Our results show that chimerism is not associated with blood transfusion history, with respect to either the number of units of male PRBCs transfused, the time between last transfusion and death, or the mean time between transfusions and death. Concludingly, chimerism is not uncommon in organs of women with sons, and significant chimerism can occur in the absence of autoimmune disease. The present study is, at least to our knowledge, the largest so far attempting to systematically evaluate the frequency of male cells in normal organs of women who had a son. Women with sons have been studied extensively in studies on the role of chimeric cells in several autoimmune diseases,^{2,4,7,8,16,17} but in most of these studies data on normal tissues were missing.^{4,8,16,17} Therefore, it was essential to establish the frequency of these male cells in women without autoimmune disease.

In a previous study we had already found that the occurrence of chimeric cells may differ widely from organ to organ.²⁷ We also found this in the present selection of organs. An important additional finding from this study is the relatively high amount of chimerism in lungs. Interestingly, Khosrotehrani et al.³¹ found that in organs of pregnant mice, the lungs were a predilection site for chimerism. They hypothesized that after entering the maternal circulation, the first capillary bed that fetal cells encounter is in the lungs, where they are trapped. It is possible that this first organ effect remains detectable in humans even years after pregnancy.

No study was previously performed that investigated the presence of Y chromosome-positive cells in such an extensive number of organs from normal women of whom blood transfusion data for at least 10 years before decease were known as well as the cause of death. Data on the sensitivity of the in situ hybridization and area of the tissue surveyed were also included. Our study has several limitations. We included organs obtained at autopsy that by light microscopy had no histological lesions. The patients had no clinical histories of autoimmune disease, but we cannot exclude that a minority of the organs that we investigated had undetectable, subclinical disease. In some cases, the cause of death directly affected the organs studied, however, we never included tissue specimens with apparent disease. For instance, in the case of bronchopneumonia we investigated a tissue specimen from the lung outside the area of inflammation. The patients were relatively old, meaning that the status of chimerism in younger women might be different. Blood transfusion data were known for at least 10 years before time of death. It is known that chimeric cells may persist even longer than 10 years.²¹ Therefore, we cannot exclude the possibility that in some cases, the chimeric cells may have been derived from blood transfusions given more than 10 years before death. In the present study we did not find a relationship between different variables regarding blood transfusion history (e.g. number of units transfused) and the presence of chimerism. This is an interesting finding, because previous studies have shown that in trauma patients that received blood transfusions, donor-derived DNA can be detected even months after transfusion, making blood transfusions a possible source of chimerism.^{24,25} On the contrary, in several other studies donor-derived cells were not detected after blood transfusion.^{32,33} Therefore, it may very well be that certain conditions, e.g. the underlying illness, the methods used to detect the chimeric cells, the time since transfusion, or characteristics of patient and donor, may play a role in the occurrence of blood transfusion induced chimerism. Therefore, we cannot exclude the possibility that in some individual patients, a proportion of the chimeric cells that we found were indeed derived from blood transfusion. Alternatively, our sample size may have contributed to this negative result.

Furthermore, it is difficult to relate our results to some of the previous studies on chimerism in autoimmune diseases, because they used different techniques such as

fluorescence in situ hybridization (FISH) or PCR targeting different parts of the genome^{8,30,34-36}. The sensitivity of the techniques was not investigated in every study. However, our in situ hybridization targeting the Y chromosome had a sensitivity of 58%, which corresponds to results from a number of studies using FISH,²⁸⁻³⁰ and entails that the number of chimeric cells found in our study is actually underestimated.

During pregnancy, not only fetal cells enter the maternal circulation, but also maternal cells can enter the fetus, and these cells can persist for decades as well.³⁷ Their pathogenic importance has e.g. been illustrated in neonatal lupus syndrome.³⁸ However, in contrast to fetal chimerism, maternal chimerism is characterized by an even lower number of peripheral cells with different subpopulations than in fetal chimerism, which may explain why autoimmune disease in newborn and young children occurs less frequently than in adults.³⁹

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Chimeric cells are a relatively common phenomenon in normal organs, whereas autoimmune diseases are relatively uncommon. Currently, there are three important hypotheses on the possible role of chimeric cells in autoimmune disease.^{18,19} In short, the first two hypotheses pose an inducing role of chimeric cells through either a graft-versus-host or through a host-versus-graft reaction (sometimes referred to as 'harmful chimerism'⁴⁰). The third hypothesis states that chimeric cells are essentially involved in repair processes ('helpful chimerism'⁴⁰). What are the implications of our finding chimeric cells in normal tissues for autoimmune disease in light of these hypotheses? Apparently, the finding of chimeric cells per se is not enough evidence for assuming that 'harmful chimerism' is present. Results from our study make clear that the chimeric cells we found in organs without disease did not lead to either a graft-versus-host or a host-versus-graft reaction. For these reactions to occur, other factors need to be present as well, which also becomes apparent from experimental models. For instance, a graft-versus-host like disease in mice resembling human lupus nephritis only occurs if chimeric is induced in specific mouse strains.⁴¹ Also in human SLE, an association between disease occurrence and bidirectional HLA class II compatibility between parents and offspring has been reported.²⁰ Excluding a role for 'harmful chimerism' in organs without disease, there is a possibility of the chimeric cells that we found in

the present study being the result of ‘helpful chimerism’, namely of tissue repair, in line with the third hypothesis. Tissue repair by chimeric cells can occur after disease induced injury. This was, for instance, reported in several experimental models using different injury models in multiple organs.⁴²⁻⁴⁴ Also in humans, case reports of fetal cells that had the morphology of fully mature and differentiated thyroid and liver tissue have been reported.^{5,22} Alternatively, tissue repair may take the form of tissue maintenance, by which progenitor cells replace single cells in the process of cell turnover. The patchy distribution of chimeric cells in the organs from the present study may reflect a process of chimeric cell maintenance. In experimental models, pregnancy-derived chimeric cells follow a similar, patchy distribution in tissue.³¹

To conclude, our results clearly demonstrate that Y chromosome-positive cells are relatively common in thyroid, lungs, skin and lymph nodes of healthy women who had sons. We also demonstrate that the number of chimeric cells in these organs is not related to the blood transfusion history. These data will serve as a point of reference for future studies of chimerism in autoimmune disease, transplantation, and pregnancy.

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