# MINIREVIEW

# Systemic Sclerosis: New Insights in Autoimmunity (44424)

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*Abstract.* **The strong female predilection of systemic sclerosis, especially in women after their childbearing years, and the clinical and histopathological similarities with chronic graft-versus-host disease make systemic sclerosis an interesting subject of debate. Recent studies concerning the pathogenesis of this disease demonstrated the persistence of fetal cells in the maternal circulation in a majority of female patients. How or whether microchimerism is involved in the pathogenesis of systemic sclerosis remains to be elucidated. The present paper reviews the recent findings on the subject.** [P.S.E.B.M. 1999, Vol 222]

Systemic sclerosis (SSc) is a connective tissue disease characterized by three features: 1) induration and thickening of the skin due to excessive collagen deposition; 2) abnormalities involving both the microvasculature and larger vessels (e.g., Raynaud's phenomenon); and 3) fibrotic degenerative changes in muscles, joints, and internal organs like the esophagus, intestinal tract, heart, lungs, and kidneys (Table I) (1–3). SSc has been described in all races and is global in its distribution. The incidence of SSc is approximately between 4 and 12 individuals per million per year. The 5-year cumulative survival rate is around 50%. The disease is three to eight times more common in women than men, and its onset is the highest in the fourth and fifth decades of life (4–8).

SSc is characterized by the presence of autoantibodies (i.e., antinuclear antibodies, ANA). Antibodies virtually specific for SSc include antibodies to topoisomerase I (ScL-70), a nonhistone nuclear protein, and anticentromere antibodies (1). Results of recent studies suggest that SSc is a disease of major histocompatibility complex (MHC)-associated autoantibody responses. Both the anticentromere and the antitopoisomerase I antibody responses have been linked to HLA-DQB1 alleles, although recent data suggest that certain HLA-DRB1 alleles are important for the antitopoisomerase I response and that HLA-DPB1 alleles may also have a role (9). It was proposed by Arnett (10) that certain HLA class II alleles, especially HLA-DQ, are more strongly associated with autoantibody subsets of SSc than with the disease itself. He showed, for example, that anticentromere antibodies are strongly associated with HLA-DQB1*0501 (DQ5) and DQB1*0301 (DQ7) alleles and that antitopoisomerase I antibodies occur in SSc patients with HLA-DQB1*0301 (DQ7), DQB1*0302 (DQ8), and DQB1*0601 (DQ6) alleles.

## Clinical Picture

The term scleroderma is a collective noun for different forms of sclerodermatous disorders. Systemic sclerosis (SSc), on the one side of the spectrum, involves fibrosis of both skin and internal organs. It can be classified in limited (cutaneous) SSc and diffuse (cutaneous) SSc. In limited SSc, the skin involvement is limited to the hands, feet, face, and/or forearms; in diffuse SSc, skin thickening is also present on upper arms and trunk. The acronym CREST (Calci-

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**Table I.** Involvement of Various Organs in Systemic Sclerosis

| Organ | Involved in |
|---|---|
| Skin | 90% |
| Vascular system—Raynaud's phenomenon | 80% |
| Esophagus | 80% |
| Lungs | 45% |
| Heart | 40% |
| Kidneys | 35% |
| Joints | 25% |
| Muscles | 20% |

nosis, Raynaud's phenomenon, Esophageal dysmotility, Sclerodactyly, and Telangiectasia) fits into the subclassification of limited SSc (7, 11). The other side of the spectrum is localized scleroderma (LS) or morphea. Here, the fibrotic lesions are well circumscribed and confined to the skin, and they differ from SSc in the absence of Raynaud's phenomenon and significant systemic involvement (12). A further subclassification of localized scleroderma was proposed by Greenberg and Falanga (13) in morphea, linear scleroderma, and generalized morphea.

In 1980, a subcommittee of the American Rheumatism Association (ARA) set up preliminary criteria for the classification of SSc (Table II) (2). With one major or two or more minor criteria, patients can be classified as SSc with a sensitivity of 97% and a specificity of 98%. These criteria are derived from a population of definite SSc patients and developed to allow comparability of patient groups in clinical trials, not to diagnose SSc in early stages (11). This review will concentrate on the pathogenesis and immunological mechanisms of systemic sclerosis.

## Pathogenesis of Systemic Sclerosis

Although the pathogenesis of SSc remains unknown, the altered connective tissue metabolism, vascular abnormalities, and changes in the immune system all seem to be involved. The altered connective tissue metabolism is characterized by the deposition of increased amounts of extracellular matrix components like collagen, fibronectin, and glycosaminoglycans. Tissue cultures of dermal fibroblasts of SSc patients have been found to synthesize collagen at an increased rate compared to normal skin fibroblasts (7). The study of Ihn *et al.* (14) showed that lymphokines like IL (interleukin)-2, IL-4, and IL-6 were detected in serum from patients with localized scleroderma but not in those of healthy controls. Since IL-4 is known to stimulate the synthesis of the extracellular matrix proteins by human dermal fibroblasts *in vitro* and IL-6 is known to stimulate fibroblast proliferation, these lymphokines may play a role in the development of the symptoms of localized scleroderma (14). Assuming that the same mechanisms will apply to the pathogenesis of systemic sclerosis, these lymphokines might be of importance in the pathogenesis of this disease as well. It is also suggested that growth factors such as platelet-derived growth factor and fibroblast growth factor promote a high collagen-producing fibroblast population (7).

**Table II.** ARA Scleroderma Criteria Cooperative Study: Preliminary Criteria for the Classification of Systemic Sclerosis (Scleroderma)

| | |
|---|---|
| 1. Major criterion: | • Proximal scleroderma |
| 2. Minor criteria: | • Sclerodactyly |
| | • Digital pitting scars or loss of substance of the digital finger pad |
| | • Bibasilar pulmonary fibrosis |

Evidence for vascular dysfunction is demonstrated by the presence of Raynaud's phenomenon as an indicator of vasomotor instability in up to 80%–90% of SSc patients (8). Possible explanations of endothelial cell damage in SSc are exposure to endothelial cell cytotoxins produced by activated T cells or complement activation. Although vascular injury is still ill-defined, it is known to facilitate adhesion and transvascular migration of mononuclear leukocytes (mostly $CD4^+/CD8^-$ T cells and monocytes) (15). A possible role of integrins and cell adhesion molecules (16) in the pathogenesis of the mononuclear cell infiltration and fibrosis of the skin that occurs in SSc was investigated by Sollberg *et al.* They found $\beta_1$ and $\beta_2$ integrins and ICAM-1 and ELAM-1 present in SSc skin but not in normal skin. They concluded that these adhesion molecules may be responsible for the homing of pathogenetic lymphocytes to skin and their adhesion to endothelial cells, and that this sequence of events may be of critical importance in the development of the early pathologic changes of SSc (17).

Changes in the humoral immune system are characterized by the detection of several antinuclear autoantibodies, as already described above. Although the regulated production of autoantibodies is a normal event, these regulatory mechanisms are in some way deflected in SSc, causing an abnormal autoimmune response to self-antigens (1). A decreased number of T cells and especially suppressor T cells in the peripheral blood are concerned with aberrant cell-mediated immune responses (7).

## Classical Autoimmune Theory

Until recently it was thought that the aberrant humoral immune response in SSc patients, the production of antibodies against self nuclear antigens, was the key factor in the development of an autoimmune disease. An abnormal autoimmune response to self-antigens implies that there is a loss of immune tolerance (1). In general, several theories have been proposed to explain the loss of tolerance in autoimmune disease. The first explanation involves the theory of the sequestered (inaccessible) antigens. An immune reaction develops to a self-antigen not normally present in the circulation. For example, in the case of tissue injury, tissue antigens, which are normally sequestered, are released into the circulation. Nevertheless, there is little evidence that these sequestered antigens are pathogenic and induce generalized injury. The second explanation holds the theory of

an abnormal T-cell function. Autoimmune reactions have been claimed to develop as a result of abnormalities in the T-lymphocyte system. A numerical decrease in suppressor T cells might be a direct cause of the break in tolerance in autoimmune diseases. On the other hand, an enhanced helper T-cell function can cause autoimmunity in two different ways. First, by altering the antigen so that the helper T cell is activated and triggers the B cell. A second mechanism by which the helper T-cell tolerance is overcome involves antibodies against foreign antigens that cross-react with self-antigens (a process known as biologic mimicry). Here, helper T cells function correctly and do not induce true autoantibody formation. A third theory involves polyclonal B-cell activation. Polyclonal B-cell activation, in which B lymphocytes are directly activated by complex substances that contain many antigenic sites (e.g., bacterial cell walls and viruses) postulates another mechanism explaining the loss of tolerance. There is some evidence that polyclonal B-cell activation may be involved in the formation of autoantibodies (1).

Central to the concept of autoimmunity is a breakdown in the ability of the immune system to differentiate between self- and nonself-antigens. This breakdown has probably occurred somewhere in the development of T cells: aberrant positive and/or negative selection in the thymus can generate potentially autoreactive T cells. In the positive selection, which takes place at the thymic cortical epithelial APCs, those precursor T cells that are capable of interacting with class I or class II MHC molecules, depending on whether cells are $CD8^+$ or $CD4^+$, are selected to survive. The process of negative selection involves the signaled death of precursor T cells that can bind well to self-peptides on self-MHC molecules on the thymic dendritical cells (medulla) (18, 19). Marrack and Parker (18) proposed that receptor occupancy, a function of the intrinsic affinity of the T-cell receptor for its peptide/MHC ligand and the ligand density on the selecting cells, may determine the fate of developing T cells in the thymus. They called this the avidity hypothesis. Besides deletion of false reactive cells, thymic selection processes can cause cells to become (reversibly) anergic (i.e., functionally inactivated due to the absence of a second costimulatory signal).

## New Concepts in Autoimmunity

What made investigators doubt the initial concepts regarding autoimmunity? Two features concerning SSc are worthwhile to discuss in regard to the pathogenesis of SSc: the clinical similarities between SSc and chronic graft-versus-host disease (GVHD) and the strong female predilection of SSc with an increased incidence after the childbearing years (20).

Different forms of GVHD can be discerned. Acute GVHD is a significant complication of allogeneic bone marrow transplantation and has its onset within 100 days after transplantation. It occurs in up to 80% of patients receiving allogeneic bone marrow transplants in varying degrees of severity (21). The principal organs affected by acute GVHD are the skin, the liver, and the gastrointestinal tract (22). Common signs of acute GVHD are fever, rash, anorexia, nausea, vomiting, and diarrhea. This type of GVH-reaction is believed to be caused by the presence of mature immunocompetent T cells in the histoincompatible as well as in the histocompatible (23) hematolymphopoietic graft. Donor T cells get activated by recognition of recipient APCs, recruit other immune cells, and damage tissue. In most bone marrow transplantation patients, their own hematopoietic system is eradicated or at least extremely suppressed by the preparative regimen, and the patient is not able to defend himself or herself against a response of donor cells and reject alloreactive donor cells. Subsequently, donor cells are able to attack the immunologically defenseless recipient (24–26).

Chronic GVHD is a late disabling complication after allogeneic bone marrow transplantation. It can develop as a consequence of acute GVHD after treatment, or it can develop *de novo* (27). It is characterized by features like sclerosis and atrophy of the skin, sicca syndrome, pigmentation disorders, and immunodeficiency (28). This form of GVHD has a typical sclerodermatous character. The pathogenesis of chronic GVHD has been thought to differ from acute GVHD, although it is not fully understood. Gamma interferon (IFN-$\gamma$) seems to play a central role in the increased collagen deposition (29–31). Roles of other cytokines like TNF (tumor necrosis factor)-$\alpha$, IL-1, and IL-6 have also been suggested in the pathogenesis of chronic GVHD (32). Associations have also been made between certain viral infections like herpes zoster viral infection (33) and cytomegalovirus infection (34), and the occurrence of chronic GVHD.

GVHD is also increasingly recognized after solid-organ transplantation (26). For example, besides the development of GVHD after transplantation of the richly vascularized spleen, GVHD also developed after liver transplantation (35). GVHD can also occur after transfusion of leukocyte-containing blood to immunocompromised recipients or to immunocompetent recipients (26) sharing HLA antigens with the transfusion donor. Examples of this so-called transfusion-associated graft-versus-host-disease (TA-GVHD) are blood transfusions or intrauterine transfusions, mostly due to administration of unfiltered and unirradiated blood. TA-GVHD is of acute onset, and it is a fatal condition in more than 90% of cases, mainly because of bone marrow hypoplasia (25). Progression to chronic GVHD with a sclerodermatous character does not occur.

The increased incidence of SSc in women after the childbearing years indicates that the onset of SSc might be pregnancy-related. Therefore, further consideration has been given to the immunologic changes that occur during this particular period. Pregnancy presents an immunologic challenge to a woman since half of the genes of the fetus are derived from the father (36). Besides HLA-G, classical HLA antigens are not expressed on trophoblast cells at the

maternal-fetal interface (22). However, fetal leukocytes, which express classical HLA antigens, are able to penetrate the maternal circulation (6). Indeed, Bianchi *et al.* (37) confirmed that male fetal progenitor cells can persist in the maternal blood for as long as 27 years after birth. Their data demonstrated the presence of fetal $CD34^+$ or $CD34^+$ $CD38^+$ cells, acquired from a prior pregnancy, in the maternal circulation. The persistence of fetal cells in the maternal circulation was an important concept for both the studies of Nelson and Artlett (5, 38).

By Artlett *et al.* (6) it was proposed that SSc may be a form of chronic graft-versus-host-disease (GVHD) induced by the presence of persisting fetal cells. To test this hypothesis they analyzed the inheritance of HLA class I and class II haplotypes in the families of 37 SSc patients and 42 control individuals and found that 70.2% of SSc patients had HLA class II alleles compatible with either their offspring or their mother, compared with only 21% of control individuals. Therefore they proposed that SSc may, indeed, be a form of chronic GVHD caused by fetal (or maternal) cells that have crossed the placenta during pregnancy and have remained unrecognized by the mother due to class II HLA compatibility. The underlying mechanism causing GVHD in this example might be the same as in TA-GVHD. In immunocompetent patients, TA-GVHD is caused by transfusions from an HLA-homozygous donor, who can be a related or an unrelated donor, to an HLA-heterozygous patient who shares a haplotype (39). The donor recognizes the patient's other HLA haplotype, but the patient does not recognize the donor haplotype as foreign (39–41). Similarly, in the example described above, the HLA-homozygous fetus recognizes the HLA-heterozygous mother. Subsequent activation of these fetal cells by as yet unknown stimuli might result in the development of systemic sclerosis (6).

The previously described hypothesis about fetal cells causing SSc might explain the clinical and histopathological similarities between SSc and chronic GVHD. Furthermore, Artlett and coworkers (6) proposed that masking of HLA antigens contributed to the tolerance of the fetus by the maternal immune system and to the establishment of microchimerism [i.e., the presence of donor (fetal) cells in the recipient (mother)]. Microchimerism may enable the patient with SSc to display continued tolerance of the pathogenic fetal cells that have crossed the placenta. As a result, these foreign cells are able to maintain a long-lasting immune attack on the host (6).

Nelson *et al.* (38) investigated whether microchimerism was involved in the pathogenesis of scleroderma and whether HLA compatibility was associated with later development of scleroderma in the mother. The researchers used quantitative polymerase chain reaction (PCR) to amplify a Y-chromosome-specific sequence in whole peripheral blood in female SSc patients and controls. Significantly more women with SSc had made DNA in their circulation when compared with controls. Furthermore, SSc patients had significantly higher amounts of male cell DNA equivalents than controls. Some SSc patients had concentrations of male DNA higher than those found in most pregnant women. The increased microchimerism in SSc patients could be a secondary phenomenon to the disease process, although the findings do not show a direct causal link between microchimerism and disease pathogenesis. However, the finding that HLA class II compatibility at the paternal chromosome of a child was more common for scleroderma patients than for controls supports the possibility that microchimerism may be involved in the pathogenesis of scleroderma.

At the same time, Artlett *et al.* (5), independently from the other research team, provided similar evidence. They used PCR and fluorescence *in situ* hybridization (FISH) to identify Y-chromosome DNA sequences in blood and skin from female SSc patients and controls. Y-chromosome sequences were found in DNA from peripheral-blood cells in 46% of SSc patients, as compared with 4% in normal women. In skin-biopsy specimens, this distribution was 59% versus 0%. They concluded that fetal antimaternal graft-versus-host reactions may be involved in the pathogenesis of systemic sclerosis in some women. It was hypothesized that a subsequent event, such as an environmental exposure (viral, chemical, or other) can activate resident fetal cells in the maternal circulation, initiating a cascade of events that results in systemic sclerosis.

## Immunological Aspects of Pregnancy

Pregnancy can result in two different outcomes concerning the immunological features between mother and child. On the one hand, it has already been known that the fetus can immunize the mother, since maternal antibodies against paternally derived HLA that are inherited by the fetus are detectable in the circulation of 20% of primigravidae and 40% of multigravidae (36, 42). Some transplantation centers refrain from using a woman donor who is mismatched for HLA antigens that are shared by her husband. Due to previous pregnancies, the woman might be sensitized against these paternal antigens. To investigate this in more detail, Bouma *et al.* (43) studied whether pregnancy can prime the maternal immune response directed toward paternal HLA antigens. Their results suggested that only those individuals who share a paternal HLA antigen against which a mother has formed HLA-specific alloantibodies should be excluded from organ donation. If no alloantibodies are formed, the mismatch is considered permissible. On the contrary, maternal cells are able to penetrate the fetal circulation and immunize the fetus. This is evidenced by cutaneous manifestations of GVHD due to maternofetal lymphocyte engraftment in patients with severe combined immunodeficiency (SCID). It is, however, not uncommon for SCID patients to develop GVHD, since it is recognized that 25% of all newborn infants with SCID receive maternal T lymphocytes *in utero* through the placenta (44).

However, pregnancy can also result in a mutual state of tolerance between mother and fetus. Although the fetus ex-

presses paternally inherited antigens that are allogeneic to the mother, it is not normally rejected by the mother. In other words, the fetus escapes the mother's immune system, and the mother becomes tolerant for her fetus. One explanation for lack of rejection is that the uterine decidua may be an immunologically privileged site that is not accessible to functional T cells. This explanation is supported by the observation that pregnant mothers are able to recognize and reject allografts syngeneic to the fetus, placed at extrauterine sites without compromising fetal survival. The basis of this immunologic privilege is clearly not a simple anatomic barrier because maternal blood is in extensive contact with the trophoblast. Rather, the barrier is likely to be functionally inhibitory, since cultured decidua cells have been shown to inhibit macrophage and T cell functions directly, perhaps by producing inhibitory cytokines, such as transforming growth factor-β. Some of these inhibitory decidual cells may be resident suppressor T cells, although the evidence for this proposal is not convincing. A second explanation for fetal survival is that extravillous trophoblast cells fail to express paternal HLA molecules (45). Human trophoblast cells may only express a nonpolymorphic class I-like molecule, HLA-G, but no class II HLA molecules. The expression of the HLA-G gene during pregnancy may prevent fetal graft rejection. It is believed that the α1-domain of HLA-G may inactivate natural killer cells, the predominant immunoreactive effector cell found in the uterus, through interaction with killer inhibitory receptors (KIR) (42, 46). However, even if trophoblast cells did express classical HLA molecules, they may lack costimulator molecules and fail to act as antigen-presenting cells (22). On the contrary, the fetus can also become tolerant of maternal antigens without generating an alloimmuneresponse (42). Claas *et al.* (47) showed in a survey that 50% of patients who had received up to 50 transfusions did not form antibodies against the noninherited maternal HLA antigens (NIMA). In other words, during pregnancy the fetus had become tolerant to these NIMAs. These data indicate that a human equivalent of actively acquired long lasting tolerance has been identified (42, 47). The study of Zhang *et al.* (48) showed that the child can develop a more pronounced tolerance toward the mother after breast-feeding. They found that breast-feeding specifically downregulated the CTL alloresponse of children to their maternal but not to their paternal HLA alloantigens (48). These findings are partially supported by Keever *et al.* (49). They proposed that the alloreactive potential of cord blood cells may be somewhat decreased. However, since cord blood is not deficient in alloreactive precursor cells, allogeneic cord blood transplantations can cause GVHD.

The presence of fetal cells in the maternal circulation early in gestation is a potentially valuable marker for prenatal diagnosis of the current pregnancy. For example, fetal cells in maternal blood could be used for noninvasive determination of Rhesus D antigen status in fetuses whose mothers are Rh(D) negative (50). Also, significantly higher amounts of fetal cells are present in the maternal circulation when the fetus had Down syndrome. These characteristics offer valuable tools for prenatal screening (50).

## Possible Explanations for the Persistence of Fetal Cells in the Maternal Circulation

What mechanisms are responsible for fetal cells persisting in the maternal circulation and maintaining a state of tolerance until a subsequent stimulus? The persistence of fetal cells in the maternal circulation is believed to be mediated by the sharing of specific HLA antigens between mother and fetus (36). The study of Lagaaij *et al.* (51) already showed that allograft survival after pretransplant blood transfusion is significantly higher among transfusion recipients who share at least one HLA-DR antigen with their donors than among those recipients who are mismatched for both HLA-DR antigens. They speculated that host T cells, which recognize class II antigens on the leukocytes of the blood donor, are actively downregulated. In the case of pregnancy, if the fetus is homozygous for one of the mother's haplotypes, there is a risk that the fetal leukocytes are not recognized by the mother. Therefore, the mother cannot recognize foreign HLA-antigens on fetal cells and consequently cannot develop an immune response (25, 52). Fetal cells can persist in the maternal circulation until a subsequent event triggers a graft-versus-host reaction.

However, since there is little chance that fetus and mother completely share HLA-antigens, there must be another mechanism to achieve tolerance for fetal cells. According to Wood (53) tolerance is defined as a specific state of unresponsiveness to alloantigens, accomplished by central (thymus) and peripheral (blood, tissues) mechanisms (22). Five nonmutually exclusive hypotheses have been proposed to explain the induction of tolerance to alloantigens. These are, in broad terms, deletion, anergy, ignorance or helplessness, exhaustion, and suppression (especially to maintain tolerance) (53). In this context the term microchimerism is worthwhile to be mentioned. However, it is far from clear how, or indeed whether, microchimerism results in tolerance of, for example, human organ grafts. Elwood *et al.* (54) suggested that haemopoietic microchimerism has been identified in recipients of solid-organ transplants and is thought by some to be critical for the development and maintenance of immunological tolerance. Since the level of detectable microchimerism varies over time, it is of no predictive value for the progress of the disease. However, the mechanisms concerning tolerance induction and maintenance are interrelated, nonmutually exclusive and not completely elucidated.

## Mechanisms of Tolerance Versus Autoimmunity

Adams and Hutchinson (55) proposed three mechanisms by which low levels of donor cells could induce tolerance to an allograft: 1) induction of anergy in recipient T cells to which antigens are presented by nonprofessional

antigen-presenting cells (inability to provide costimulatory molecules like CD28); 2) presence of donor cells that can inhibit recipient cytotoxic lymphocyte activity against donor antigens (veto cells); and 3) persistence of donor cells or antigens in the host thymus that lead to the deletion of host alloreactive T cells. Are the complementary mechanisms applicable to the loss of tolerance in autoimmune diseases? In other words, what mechanisms are responsible for a microchimeric cell population to contribute to the development of an autoimmune disease? The answer to this question is far from elucidated. Three mechanisms have been proposed by Nelson *et al.* (38): 1) fetal cells, which are primarily sequestered in affected tissues, could act as direct effectors of damage to host tissues and initiate a graft-versus-host reaction; however, low concentrations of fetal cells were found in the maternal circulation, which argues against a direct effector role for these cells; 2) a small population of nonhost cells (or peptides) could start a process by which subsequent damage is caused by host cells (these foreign cells are a persistent stimulation for the mother's immune system, subsequently triggering an autoimmune reaction); and 3) a small population of nonhost cells (veto cells) could downregulate host immunoregulatory cells, which would allow damage by autoreactive host cells (56). Since the alloreactivity to third party autoantigens is not inhibited by veto cells (57), destruction of self can be accomplished (20, 58).

Tolerance cannot be maintained after pregnancy in the case of SSc. There are several ways in which maternal and fetal antigens can play a role in triggering the maternal immune system. First of all, maternal antigens can either be processed by persisting fetal cells and presented to maternal T cells (indirect recognition), or the mother can recognize fetal peptides directly on the fetal MHC molecule (direct recognition). Furthermore, fetal antigens can be processed by maternal antigen-presenting cells. If these peptides are to a large extent homologous to maternal auto-antigens, these peptides could initiate a maternal auto-immune response. This phenomenon, in which fetal peptides mimic maternal antigens is called molecular mimicry. Therefore, due to an immune response to a persisting allogeneic fetal stimulus, one might better consider SSc as an alloimmune response.

In the past it has been suggested that the age-specific incidence of autoimmune diseases and the level of female sex hormones might be correlated. However, Nelson stated in her review that the levels of sex hormones do not provide an explanation for the female predilection to autoimmune disease. For example, the incidence of rheumatoid arthritis (RA) continues to rise with age at least into the seventh decade of life (8), thus lacking any correlation with the time when female sex hormone levels are high. Furthermore, exogenously administered sex steroids do not have similar effects on different autoimmune diseases or on susceptibility to disease. Finally, although sex steroid levels increase during pregnancy, pregnancy has dissimilar effects on different autoimmune diseases; for instance, pregnancy commonly induces remission of RA but not of systemic lupus erythematosus (SLE) (36).

## Points of Discussion

The theory described above regarding pregnancy-related GVHD does not provide an explanation for the occurrence of scleroderma in men and nulliparous women. Other potential sources of microchimerism in such individuals include blood transfusion or engraftment of haematopoietic stem cells from a twin (38). Another potential source could be microchimerism of maternal origin since maternal cells can traffic to the fetal compartment and have been detected in up to 40% of umbilical-cord blood samples (59). Moreover, more than one pathway can result in a similar clinical end point, as evidenced by scleroderma-like changes following exposure to vinyl chloride or epoxy resins or treatment with drugs such as pentazocine and bleomycin (7, 36).

Nelson's findings were cited by Welsh (60). He stated that if chimerism is assumed to be true, the possibility that a form of GVHD induces scleroderma cannot be taken as confirmed. In other words, although there are peripheral similarities between scleroderma and GVHD, there is no proof that any transplant recipient has developed real systemic scleroderma and that scleroderma is not an apparent risk after allogeneic cell transfer. Indeed, Rouquette-Gally *et al.* (61, 62) found that the biological autoimmune (autoantibody) profile in chronic GVH patients was not comparable to that of spontaneous SSc, suggesting that they have different pathophysiological mechanisms. However, since autoantibodies have been found in all patients with chronic GVHD, this is probably caused by the persistence of an allogeneic stimulus. Welsh also questioned Nelson's sensitive PCR method because of the risk of false-positive results due to male Y-like sequences elsewhere in the genome. Assuming that the false-positive rates would be similar in controls and patients, this problem can be neglected. However, the determination of chimerism proposed a greater risk since certain amplicons would be incorrectly assigned as alleles because of sequence homology with self alleles or pseudo gene sequences (60). Indeed, it is the author's opinion that a comment can be made, pointed on the sensitivity of the PCR and FISH methods. When comparing the detection methods of Nelson *et al.* (PCR) and Artlett *et al.* (FISH), the sensitivity of detection varies. For example, Nelson *et al.* claim to detect one male cell DNA equivalent in 16 ml of whole blood. On the other hand, Artlett *et al.*, using FISH, were able to detect 2 Y-chromosome-containing cells per 3000 cells, enriched either for CD3 T cells or for CD14 and CD45 cells. The difference between these detection limits varies by at least a factor of 1000.

Aractingi *et al.* (63) pointed out in their comment that there are clinical, histological, and immunological differences between SSc and GVHD. They proposed that if in-

deed chimerism plays a part in SSc, as in GVHD, one may suspect that other yet unidentified factors will also contribute to its pathogenesis, leading to these differences. Recently, Connolly and McCalmont (64) commented on the study of Artlett *et al.* They stated that the dissimilar clinical and pathological features of GVHD and SSc raise questions about the use of GVHD as a model for SSc. For example, fibrosis and Raynaud's phenomenon are unusual in patients with GVHD but occur in a majority of patients with SSc. Liver involvement is common in GVHD but rare in SSc (64). Finally, GVHD responds to several immunosuppressive drugs, whereas in the case of SSc, conflicting results exist about the beneficial effects of these drugs. Chosidow *et al.* (28) added that differences between SSc and sclerodermatous GVHD concern the initial location of the sclerosis, the extent of visceral involvement, and the antibody patterns, (i.e., SSc-specific anticentromere and antitopoisomerase I antibodies are absent in sclerodermatous GVHD).

What can be said about the incidence of SSc in the general population regarding the findings described in this review? Since the amount of HLA sharing during pregnancy is around 20% (6) and the persistence of fetal cells in the maternal circulation after pregnancy is estimated to be between 1%–75% (5, 37, 38, 65, 66), this would implicate that potentially millions of women worldwide are at risk of developing SSc. However, since the actual incidence is much lower, other predisposing factors have to play a major role. Genetic and/or environmental factors might be involved in the susceptibility for developing SSc.

A recent report in *Lancet* about the presence of fetal DNA in skin of polymorphic eruptions of pregnancy supports the association between pregnancy-related chimerism and skin disorders. Aractingi *et al.* (67) showed that male DNA was detected in dermis or epidermis from skin lesions of six of ten women with this cutaneous disorder of pregnancy. They proposed that the skin could be a homing tissue for chimeric cells during gestation.

## Future Perspectives

Future studies are of the utmost importance to confirm or exclude the recent results concerning the persistence of fetal cells in the circulation of female SSc patients. Further investigations will be based on unravelling the immunological mechanisms and the role of microchimerism in the pathogenesis of SSc. If the presence of fetal cells can be confirmed in future studies, it is important to determine the alloreactive potential of the fetal and maternal cells against each other and selected HLA-compatible and HLA-incompatible third party controls. Several aspects regarding the pathogenesis of SSc need to be resolved before a complete understanding of this disease can be accomplished.

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