Systemic lupus erythematosus: pathogenesis, diagnosis, and treatment

Wilhelmus, S.

1

General Introduction

Introduction

Systemic lupus erythematosus (SLE) is a potentially devastating autoimmune disease which can involve practically every organ system. SLE has an overall incidence ranging from 1.6 to 21.9 cases per 100 000 per year and a prevalence ranging from 7.4 to 159.4 cases per 100 000, varying considerably by ancestral group.[1] SLE affects mostly women of reproductive age. In contrast, in men, the incidence ranges from 0.14 to 2.5 cases per 100 000 per year and the prevalence from 0 to 52 cases per 100 000.[2] Up to 20% of all cases begin in childhood. The female predominance is not as outspoken in childhood-onset SLE as it is in adult-onset SLE.[3] Patients with childhood-onset SLE are more likely to have neurologic and renal involvement than patients with adult-onset SLE, and to accrue more renal damage.[4] Renal and neurological involvement are both considered to be severe manifestations of the disease. Approximately 20 to 60% of SLE patients develop renal involvement in the course of their disease[5] with the highest risk of renal disease and renal failure in young black women.[6 7] Lupus nephritis (LN) is associated with considerable morbidity and poor survival, in particular in patients who develop end-stage renal disease (ESRD) and require renal replacement therapy.

The diagnosis, treatment, and pathogenesis of SLE are intricately linked. The diagnosis of SLE can be difficult because of the many different faces of the disease. These many faces are also present within one of the disease manifestations: LN. The histological picture of LN varies greatly, but a correct diagnosis of the type of LN is essential for the choice of treatment. The question also is if different classes of LN have a different pathogenesis, or if they are part of the same spectrum. Familial LN often presents at an early age and appears more severe than sporadic LN. There may be an underlying difference in pathogenesis and possibly genetics between familial and sporadic LN, which can be used in studying the pathogenesis of LN. Further insight into the pathogenesis of SLE and LN may lead to new targets for therapy in the future.

Diagnosis

SLE

SLE can involve practically all organ systems. Therefore, patients can present with a wide range of symptoms. Not all symptoms are necessarily present at the same time. These factors can make diagnosing SLE complex. The first classification criteria were published by Cohen *et al.* in 1971.[8] The presence of four of 14 criteria was required to classify a patient as

having SLE. During the following decades new insights led to revisions by the Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology (ACR) in 1982[9] and 1997.[10] For the purpose of identifying patients in clinical studies, a patient was considered to have SLE if any four of 11 criteria were present, serially or simultaneously, during any interval of observation. These 11 criteria were a malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurologic disorder, haematological disorder, immunological disorder and an abnormal anti-nuclear antibody (ANA) titer. Recently, the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria were introduced, which were validated in a cohort of 690 patient scenarios including control patients with RA, undifferentiated connective tissue disease, primary antiphospholipid syndrome, vasculitis, chronic cutaneous lupus erythematosus, scleroderma, Sjögren's syndrome, myositis, psoriasis, fibromyalgia, alopecia areata, and sarcoidosis. [11] In the SLICC system, the criteria have been distributed over 11 clinical and six immunological criteria. In order to classify a patient with SLE, at least four criteria with at least one clinical and one immunological criterion must be present. Also, biopsy-proven nephritis compatible with SLE in the presence of ANAs or anti-dsDNA antibodies will also classify the patient as having SLE.

Lupus nephritis

The renal biopsy plays an important role in the management of patients with SLE. Renal manifestations may be the first sign of SLE and the renal biopsy may then aid in diagnosing the patient. Furthermore, in both these newly diagnosed patients and patients already diagnosed with SLE, the renal biopsy is instrumental in determining the type and extent of LN, as this cannot be accurately assessed on the basis of clinical manifestations.

Electron microscopy

Instrumental in the pathology of LN are immune deposits. These immune deposits can be visualized with immunofluorescence techniques (discussed below) and electron microscopy (EM). On EM, immune deposits are electron dense and can be present in the mesangium, subendothelially, intramembranous and subepithelially. Often in LN, immune deposits are found at more than one of these locations (Figure 1, panel A, C and D). The size and frequency of these deposits are extremely variable, ranging from sparse and small to abundant and large. Immune deposits are not restricted to the glomerulus and can be present along tubular basement membranes and in vessels. Another feature that may be seen by EM is the presence of tubuloreticular inclusions, which are mostly found in endothelial cells (Figure 1,

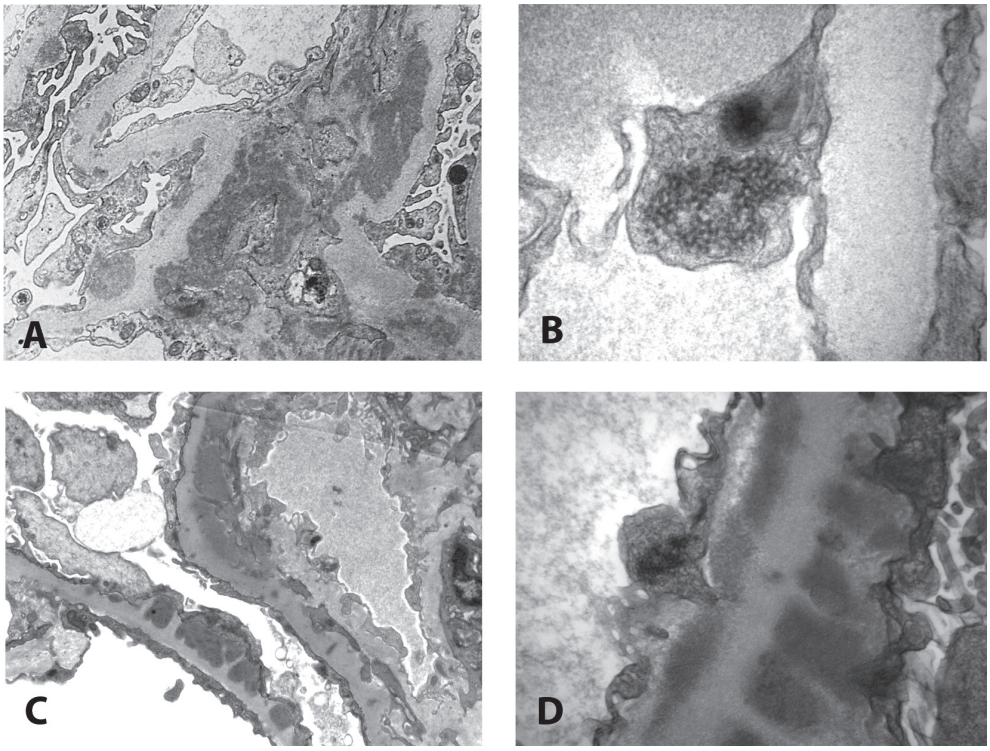

Figure 1. Examples of lesions in lupus nephritis, as seen by electron microscopy
(A) Extensive mesangial electron dense deposits. (B) Tubuloreticular inclusion in an endothelial cell. (C) and (D) Electron dense deposits at both subendothelial and subepithelial locations. This is accompanied by foot process effacement and microvillous transformation.

panel B). Although they are often present, they are not specific for SLE and may be seen in patients with HIV, other collagen-vascular diseases, renal allografts[12] and even a few healthy individuals. Finally, particularly in patients with subepithelial deposits, changes to the podocyte foot processes can be observed. These changes include foot process effacement, condensation of the cytoskeletal microfilaments and microvillous transformation.

Histology

LN has many different histological features which correspond to the location of the immune deposits seen on EM. These deposits can be visualized by EM, and by immunofluorescence techniques. In contrast to EM, immunofluorescence allows for the determination of the type of immune deposit (IgA, IgG, IgM antibodies) and of the presence of components of the complement system (C1q, C3). Often IgA, IgG, IgM, C1q and C3 are all present, which is

commonly referred to as a “full house” pattern. This pattern is highly suggestive of LN, but not completely specific as other renal diseases may occasionally show a full house pattern.[13] For assessing a biopsy with light microscopy, multiple special stains are used. Apart from the regular haematoxylin and eosin staining (H&E), special stains are used such as the Jones methenamine silver stain, the periodic acid-Schiff (PAS) stain and trichrome stain. The patterns of injury in LN can be divided into three groups. These patterns are not mutually exclusive and can occur together.

Mesangial pattern

In this pattern there is hypercellularity of the mesangium and accumulation of matrix due to the mesangial presence of immune complexes (Figure 2, panel A).

Endothelial pattern

A wide variety of lesions may be seen within this pattern. The most common feature is endocapillary hypercellularity, which causes a luminal reduction of the capillary loops (Figure 2, panel C). This endocapillary hypercellularity has two components which may vary in its contribution: endothelial cell swelling and leukocyte influx. Another feature is fibrinoid necrosis.[14] This is often accompanied by extracapillary hypercellularity, so called crescents, because there is destruction of the capillary walls causing the capillary contents to leak into Bowman’s space. This elicits an inflammatory response and proliferation of visceral and parietal epithelial cells. Crescents may also occur without fibrinoid necrosis (Figure 2, panel D). Wire loops, the light microscopical counterpart of large amounts of subendothelial immune complex deposits, can be a focal or diffuse phenomenon (Figure 2, panel B). Furthermore, a membranoproliferative pattern may occur showing cellular interposition of mesangial cells along capillary walls and duplication of the glomerular basement membrane. Finally, karyorrhexis and hyaline thrombi may be observed.

Epithelial pattern

When immune complexes accumulate on the subepithelial side of the glomerular basement membrane, new glomerular basement membrane is formed around these deposits. Because the glomerular basement membrane is black on silver stain, this newly formed basement membrane can be seen as black spikes along the outer aspect of the capillary walls (Figure 2, panels E and F). If this new glomerular basement membrane has not yet been formed, light microscopy may be normal with deposits visible only by immunofluorescence and electron microscopy.

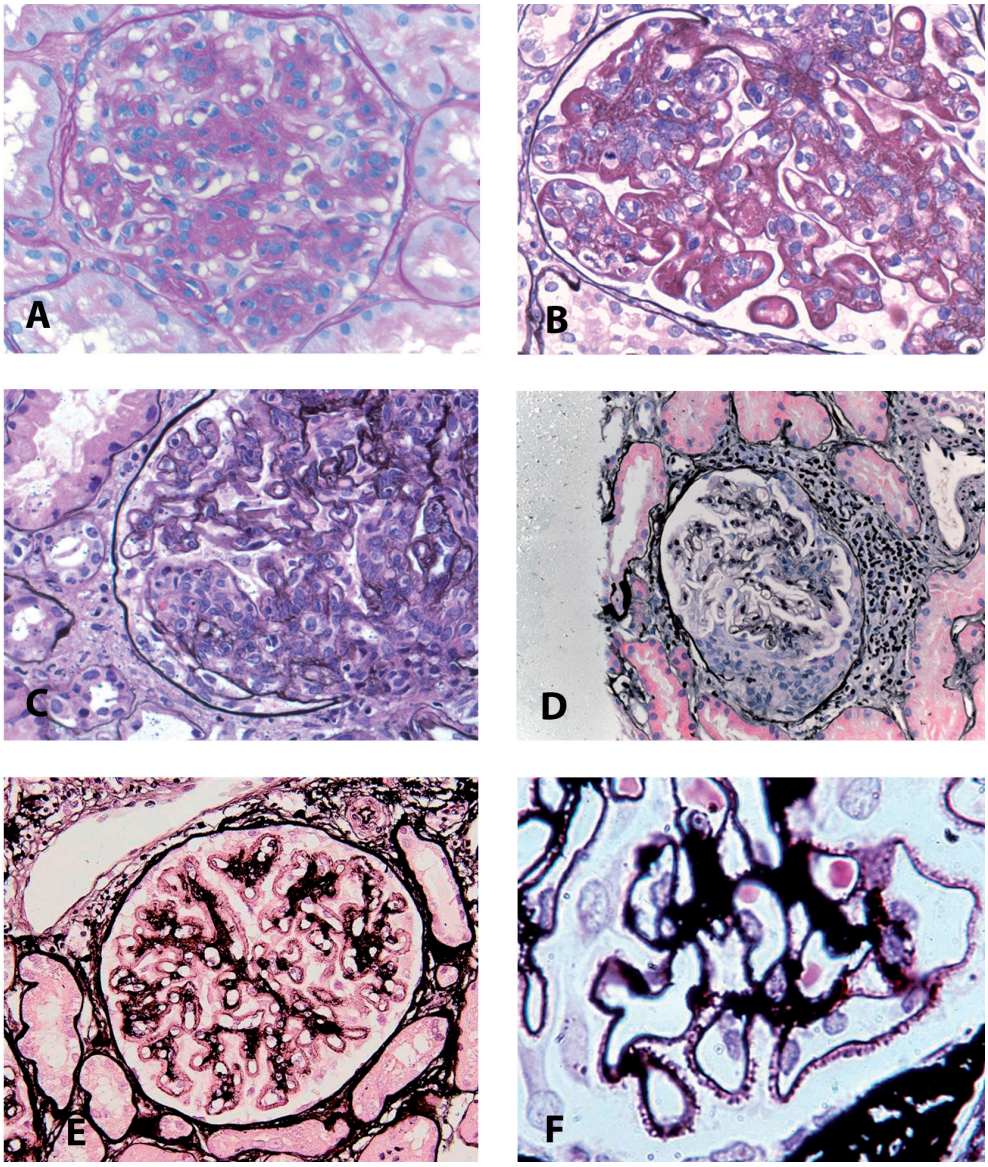

Figure 2. Examples of lesions in lupus nephritis, as seen by light microscopy
(A) Mostly mesangial hypercellularity and expansion with focal endocapillary hypercellularity (PAS stain). (B) Diffuse wire loops with mild endocapillary hypercellularity (silver stain). (C) Endocapillary hypercellularity with endothelial cell swelling and influx of inflammatory cells (silver stain). (D) Cellular crescent (silver stain). (E) and (F) Spikes along the outer aspect of the capillary walls (silver stain).

Table 1. Abbreviated ISN/RPS classification system of lupus nephritis (adapted from Weening, JASN/ Kidney International, 2004[15 16])

Class	Description
I	Minimal mesangial lupus nephritis
II	Mesangial proliferative lupus nephritis
III	Focal lupus nephritis[a]
IV	Diffuse segmental (IV-S) or diffuse global (IV-G) lupus nephritis[a]
V	Membranous lupus nephritis[b]
VI	Advanced sclerosing lupus nephritis

[a] Indicate proportion of glomeruli with active lesions, chronic lesions, fibrinoid necrosis and (cellular) crescents. [b] Class V may occur in combination with class III or IV, in which case both will be diagnosed. Indicate and grade (mild, moderate, severe) tubular atrophy, interstitial inflammation and fibrosis, arteriosclerosis or other vascular lesions.

Classification

When a diagnosis of LN has been made, the biopsy findings need to be classified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification system from 2004.[15 16] It is imperative that a biopsy is classified correctly as the class will guide further treatment of the patient. Since its introduction, the classification system has undergone several revisions and now consists of 6 classes (Table 1).

In class I and II LN changes are restricted to the mesangium. Both classes show deposits by immunofluorescence, but only in class II is there mesangial hypercellularity or mesangial matrix expansion by light microscopy. In class II LN there may be a few isolated subendothelial or subepithelial deposits present by immunofluorescence (or EM) but not by light microscopy. Class III and IV LN show light microscopic abnormalities as described above under the endothelial pattern of injury. The distinction between class III and IV LN lies in the percentage of glomeruli involved with <50% being classified as class III and ≥50% as class IV. In both of these classes it should be indicated if there are either only active lesions (A), both active and chronic lesions (A/C), or only chronic lesions (C), although the latter is a rare event. Class IV LN is further subdivided into class IV-S and IV-G depending on whether the majority of involved glomeruli have segmental (IV-S) or global (IV-G) involvement, where segmental is defined as less than half of the glomerular tuft and global as more than half of the tuft. In class V there are subepithelial deposits by immunofluorescence (and EM), and possibly also by light microscopy. In class V LN any degree of mesangial hypercellularity may occur. When class III/class IV lesions coexist with class V lesions, the classification should consist of a combination of two classes, but only if the membranous component involves

more than 50% of the tuft in more than 50% of glomeruli. Finally, class VI LN is designated when ≥90% of glomeruli show global glomerulosclerosis, but only if there is clinical or pathological evidence that the sclerosis is attributable to LN. Furthermore, there should be no evidence of active nephritis. In addition to the class the pathology report should include details on tubulointerstitial and vascular lesions.

Treatment

After a diagnosis of SLE has been made, a treatment strategy can be devised. The treatment strategy depends on which organs are involved and to what extent. In the kidney, the class of LN plays a central role in the choice of treatment.

SLE

Treatment of SLE, without major organ involvement, consists of glucocorticoids, antimalarials (hydroxychloroquine), non-steroid anti-inflammatory drugs and, in severe, refractory cases, immunosuppressive agents. SLE patients may be at increased risk for several co-morbidities, including treatment-related morbidity. These include (urinary-tract) infections, hypertension, dyslipidaemia, diabetes mellitus, atherosclerosis, coronary heart disease, osteoporosis, avascular bone necrosis and certain types of cancer. Although there is no evidence that screening for co-morbidities will improve outcome, a high index of suspicion and diligent follow-up is recommended. Apart from treatment of these co-morbidities, when appropriate, preventive strategies may be considered such as low-dose aspirin in adult patients receiving glucocorticoids, in patients with anti-phospholipid antibodies, and in patients with at least one traditional risk factor for atherosclerotic disease.[17]

Lupus nephritis

Class III and class IV LN, and under certain circumstances class V LN, require aggressive treatment. Corticosteroids were the first available treatment for LN, and have since been an integral part of treatment. Treatment is comprised of two phases; induction and maintenance. The aim of the first is to achieve a meaningful renal response. The goal of maintenance is to consolidate the renal response and prevent renal flares. In the eighties and nineties of the previous century, NIH (National Institute of Health) studies demonstrated the added benefit of cyclophosphamide in the induction phase.[18-20] Cyclophosphamide is an alkylating agent thereby interfering in DNA replication. Later, mycophenolate mofetil (MMF) was shown to be equally effective.[21]

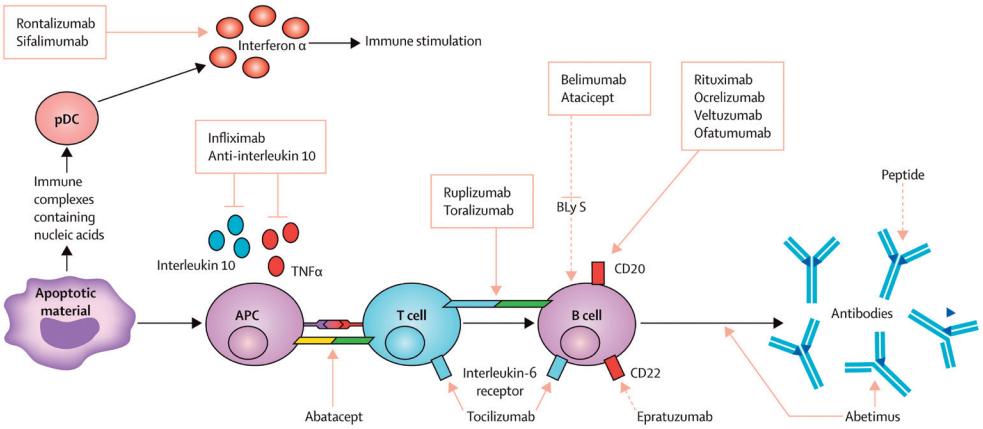

Figure 3. Targeted biological agents available and in present or previous clinical trials of systemic lupus erythematosus (reprinted from Murphy, Lancet, 2013[22], with permission from Elsevier)
The biological agents available all interact with the immune system. Some by immune stimulation through interferon a, others by targeting cytokines affecting antigen presenting cells. T cells interactions with antigen presenting cells and with B cells are also targeted, as well as the B cells and produces antibodies. Only belimumab (anti-BLyS) has so far been proven effective in SLE.
pDC, plasmacytoid dendritic cell; BLyS, B-lymphocyte stimulator; TNFα, tumor necrosis factor α; APC, antigen-presenting cell.

In the maintenance phase, treatment consists of either azathioprine, or MMF. MMF and azathioprine both inhibit purine synthesis, albeit by a different mechanism. Purine synthesis is important in the proliferation of B and T cells. In both the induction and maintenance phase, corticosteroids remain the backbone of treatment, although a phase 3 open-label multicenter investigator-led clinical trial (RITUXILUP, NCT01773616) is currently investigating a treatment strategy without steroids.

Most of the above mentioned drugs have serious possible side effects necessitating the search for effective drugs with less (severe) side effects. As more becomes known about the pathways involved in the pathogenesis of SLE and LN (discussed later), more targeted approaches are being developed (Figure 3).[22 23] For LN these drugs have not yet been proven effective. For the treatment of systemic disease belimumab, an anti-BLyS (B-lymphocyte stimulator) antibody, has been proven to be effective and is now registered for treatment of the disease.[24 25]

Pathogenesis

As discussed above, SLE may have many faces clinically. This complicates research into the pathogenesis of this complex and multifactorial disease even further. Although there are

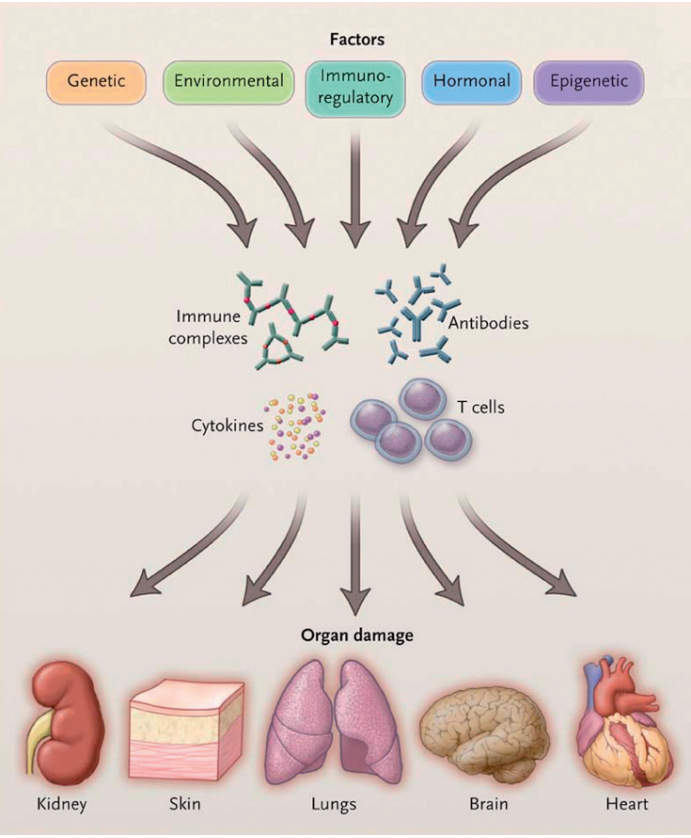

Figure 4. Factors involved in the pathogenesis of systemic lupus erythematosus (reproduced with permission from Tsokos, NEJM, 2011[39], Copyright Massachusetts Medical Society)

still many unknowns concerning the pathogenesis of the disease, substantial progress has been made in the last decades.

SLE

A multifactorial disease

Many factors contribute to the development of SLE, including (epi)genetic, environmental, hormonal and immunoregulatory factors (Figure 4).[26]

Genetic susceptibility to SLE is inherited as a complex trait, but the genetic contribution is thought to be significant in the etiology of SLE. Disease concordance in SLE is higher in monozygotic (25-50%) than in dizygotic twins (2%) and there is a high sibling risk ratio (λs) of 20-29.[26-28] Although some single disease-causing mutations have been described, such as

in DNASE1, these are only the cause of disease in rare cases.[29] Initial studies used linkage analysis in multiplex families to identify candidate genes. More recently, multiple genome wide association studies were performed in European-derived and Asian populations identifying multiple SLE susceptibility alleles.[30-38] Meta-analyses and large replication studies have expanded these further. Although some of these loci are located in coding sequences, many reside in non-coding regions. The HLA-region holds a prominent position within these susceptibility loci. The non-HLA SLE-associated genes play a role in multiple biological pathways: dendritic cell function and IFN signaling, T and B cell function and signaling, immune complex processing and innate immunity, transcriptional regulation, and cell cycle, apoptosis and cellular metabolism.[39] Despite all the advances made in the last decade, only a small proportion of the heritability is explained by these susceptibility loci.

Environmental factors also seem to play a role in pathogenesis. Epigenetic changes such as DNA hypomethylation have been attributed to medications known to cause SLE. Furthermore, epidemiologic studies have implicated smoking and exposure to ultraviolet light as risk factors. Finally, there is evidence to suggest that viruses may trigger SLE.[39]

The strong female predominance in SLE has led to research into hormonal influences. Although they contribute to the pathogenesis of SLE, the mechanisms involved are unknown. Data from animal studies suggests that the X chromosome may also contribute independently from hormones. Furthermore, CD40 is among the genes known to contribute to SLE and this gene is located on the X chromosome.[39]

Multiple abnormalities have been demonstrated in antigen presenting cells, and T and B cell signaling and function in SLE (for review, see Konya *et al*.,[40] Bird *et al.*[41] and Orme *et al.*[42]). Neutrophils have received much attention, particularly since the discovery of NETosis (formation of neutrophil extracellular traps, or NETs). NETosis has been described as a novel mechanism of cell death in which the neutrophils extrude their chromatin to trap and inactivate pathogens. NET formation appears to be enhanced in SLE leading to an increased exposure to autoantigens and increased production of IFN-α by plasmacytoid dendritic cells (for review, see Smith *et al.*[43]).

Autoantibodies against nuclear antigens are the hallmark of SLE. These antibodies are produced by autoreactive B cells (plasma cells) and may, along with immune complexes, autoreactive or inflammatory T cells and inflammatory cytokines, initiate and amplify organ damage. The factors described above are probably all involved in the production of these antibodies, but the question remains: why are these mainly directed towards nuclear antigens? The source of chromatin, the main auto-antigen in SLE, is most likely apoptotic

and/or necrotic cells, including NETs. Apoptosis, necrosis and NETosis explain how normally inaccessible autoantigens can be released and subsequently become exposed to the immune system. The autoantigens can be modified during apoptosis, possibly facilitating the breach of tolerance. In addition, impaired removal may lead to the accumulation of apoptotic cells and debris. There is convincing evidence for clearance defects op apoptotic cells and debris in SLE (for review, see Rekvig *et al.*[44]). Possibly, antibodies are not (only) generated in response to self-DNA and chromatin, but to DNA/chromatin from chimeric cells, as chimeric cells have been implicated in the pathogenesis of SLE. Before discussing the role of chimeric cells in SLE, first the definition, sources and techniques for detection of the chimeric cells are considered.

Microchimerism

Definition

The term 'chimerism' originates from Greek mythology. It refers to the Chimaera (Figure 5), which is a monster composed of parts of more than one animal. Homer's description in the Iliad is the earliest surviving literary reference: "…. an invincible inhuman monster, but divine in origin. Its front part was a lion, its rear a snake's tail, and in between a goat. She breathed deadly rage in searing fire."[45]

Figure 5. The "Chimera of Arezzo", as displayed at the Museo Archeologico Nazionale, Florence, Italy

In medicine, the term 'chimaera' is related to the term 'mosaicism', because in human cytogenetics both connote subjects with cells of two or more chromosomally different kinds. However, a chimaera "... is an organism whose cells derive from two or more distinct zygote lineages...", whereas a mosaic "... is formed of the cells of a single zygote lineage."[46] *Micro*chimerism (Mc) refers to the presence in an individual of a *small number* of genetically distinct cells of any type, originating from a different zygote.

Sources of microchimerism

Transplantation (solid organs[47] or bone marrow[48]), blood transfusions[49] and pregnancies[50] are possible sources of (micro)chimerism, the latter being the most common. During pregnancy, fetal cells can enter the maternal circulation leading to fetal Mc (FMc) in the mother. When maternal cells cross the placental barrier to the fetus, this can lead to maternal Mc (MMc). Pregnancies of all terms, including both miscarriages and pregnancies resulting in (live) birth, may lead to Mc.[51-53] Also, undetected pregnancies have the potential to cause FMc, making research into the relationship between pregnancy and long-term FMc difficult. Several studies investigated the kinetics of Mc during and after pregnancy in healthy individuals.[53-57] It was demonstrated that Mc tended to increase with gestational age and disappeared in the months postpartum. During pregnancy, not only can fetal cells circulate in the mother, fetal cell-free DNA can be detected in the maternal circulation.[53] The quick disappearance of this fetal cell-free DNA after delivery made it the perfect candidate for the prenatal detection of genetic defects in the fetus. Prenatal diagnostics are now being employed to detect trisomy 13, 18 and 21.[58] Both FMc[59] and MMc[60] have been detected in peripheral blood multiple decades after birth in healthy individuals.

During blood transfusion, genetically distinct cells are introduced into the host. Initial studies, however, were not able to demonstrate donor leukocyte survival beyond 6 days.[61 62] Interestingly, in a study characterizing the survival kinetics of donor subsets after elective surgery, it was accidently found that the 'control group' of women with blood transfusions after traumatic injury did have multilineage persistence of male donor leukocytes for 6 months to 1.5 years after blood transfusion. In the patients with elective surgery, the donor leukocytes were cleared within 14 days after transfusion.[49] Follow-up studies have confirmed these results.[63 64] Studies in other populations, such as in an HIV-infected population as a paradigm of an immunosuppressive state,[65] and sickle cell anaemia[66] reflecting chronic transfusion risk, did not show a significant increase in Mc or durability of Mc. Importantly, women receiving peripartum transfusions for maternal hemorrhage did not show durable Mc.[67]

Solid organ transplantation itself is a form of chimerism because an organ from a different individual (zygote) is transplanted into the patient. Furthermore, these patients also have circulating donor Mc in their peripheral blood.[68] So far, it is unclear if the presence or level of Mc in peripheral blood in recipients of solid organ transplantation has an effect on tolerance induction and graft function.[68-70]

Detection of microchimerism

The earliest detection of Mc employed karyotyping in metaphase figures in lymphocyte cultures from peripheral blood samples.[50] Later, many studies used *in situ* hybridisation for the Y chromosome.[55 71] This was followed by PCR techniques, first the non-quantitative nested PCR,[59] and later the quantitative PCR (qPCR).[72] Still, mostly the detection of the Y chromosome was used in women to measure Mc, although HLA genotype disparities were also sometimes used.[60] A drawback of detecting Mc using the Y chromosome is that only male Mc can be detected. This limits research to women and to FMc. It also means that in an individual all male cells are indiscriminately analyzed together and other sources of Mc (maternal, female children, female siblings) are missed. Using HLA disparities allows for studying MMc and for the detection of Mc in men. However, the presence of Mc is possibly linked to HLA disparities, making this method less desirable. In 2002, Alizadeh *et al.*[73] described a method using insertion-deletion polymorphisms (indels) for the detection of chimeric cells. Additional indels were described by Jimenez-Velasco *et al.*,[74] as well as a number of null alleles. In the latter study a sensitivity of 10^{-5} was reached, which is equal to the sensitivity reached with the detection of the Y-chromosome.[72] Maas *et al.*[75] developed an assay using SNPs, but this assay was less sensitive. Also, it was potentially less specific than the usage of indels or null alleles to detect Mc because it makes use of only a one base pair difference. Combining sets of indels and null alleles may reach a high informativity in differentiating different sources of Mc.

The number of chimeric cells detected in various circumstances is usually very small, ranging from 1 to up to 400 cells per 10^6. Therefore, both sensitivity and specificity are of the utmost importance. A sensitivity of at least 10^{-5} is necessary to study Mc. Differences in sensitivity and specificity of the techniques used in the field make comparison of the different studies difficult. This high sensitivity also requires a very clean work flow: while preparing samples all possible contaminants should be avoided. Also, all experiments require multiple negative controls.

Microchimerism in pregnancy

Schmorl *et al.* first described fetal Mc. He found syncytial aggregates in lungs of women who died of pre-eclampsia.[76] Although women with pre-eclampsia have more syncytial aggregates in their lungs, these placenta-derived syncytial aggregates have also been shown in women with normal pregnancies.[77] Also, fetal cells were detected in peripheral blood and various organs during pregnancy.[53 78] It is, however, unclear what role these chimeric cells play in normal pregnancies. Are they an epiphenomenon or do they play a central role in the immunology of pregnancy? Conversely, it is known that maternal chimeric cells can have an effect on the immune system of the child; Mold *et al.* showed that maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero.[79]

Microchimerism in SLE

SLE mainly affects women and has a peak incidence in the reproductive years.[80] In mice, injection of parental lymphocytes in their offspring leads to a graft-versus-host response and a lupus-like disease in selected parent-to-F1 combinations.[81 82] Together, these data suggest that pregnancy-acquired Mc may be of pathogenic significance in the development of SLE. Studies investigating Mc in SLE have shown that women with SLE have a significantly higher prevalence of fetal Y chromosome-positive chimeric cells in tissue than healthy controls.[83-85] In two studies, SLE patients were shown to have male FMc in peripheral blood more frequently than controls.[86 87] However, other studies showed no differences between patients and controls.[88 89] Kanold *et al.* studied MMc in peripheral blood and did not find a difference between patients and controls.[90] However, their sensitivity of detecting chimeric cells was relatively low.

Kremer Hovinga *et al.*[91] formulated hypotheses regarding the role of Mc in SLE: *i*) Mc induces a graft-versus-host reaction; *ii*) Mc induces a host-versus-graft reaction, either directly or via cross-reactivity due to molecular mimicry; or *iii*) chimeric cells repair injured tissue. The first hypothesis is supported by the data from animal studies described above. From human studies the evidence is very circumstantial. The host-versus-graft hypothesis has more support from studies in humans, albeit also circumstantial. Anti-paternal antibodies have been demonstrated in mothers and have been shown to correlate to the presence of primed anti-paternal cytotoxic T lymphocytes.[92] Also, it was demonstrated in SLE patients that patients with LN had higher levels of Mc than patients without SLE, although overall disease activity was not correlated with Mc.[89] The presence of chimeric progenitor cells in

SLE patients lends support to the third hypothesis.[59] Also in animal studies chimeric cells were shown to have stem cells phenotypes[93] and chimeric cells have been demonstrated in bone marrow and rib sections of women with sons.[94] Furthermore, Mc was increased in animal models after injury.[95 96] Finally, in kidney biopsies of women with LN, the chimeric cells were shown to have multiple differentiated phenotypes, such as an endothelial cell.[83]

Lupus nephritis

The deposition of immune complexes in the kidney is the cause of the renal damage in LN. Depending on the location of the immune deposits, *i.e.* mesangial, subendothelial or subepithelial, different mechanisms leading to renal damage are triggered. These mechanisms involve activation of the *i*) classical complement pathway; *ii*) Fc, Toll-like and complement receptor activation; *iii*) local expression of cytokines, chemokines and adhesion molecules; *iv*) recruitment of leukocytes with pro-inflammatory effector functions; *v*) programmed death of renal parenchymal cells and reparative hyperproliferation; and *vi*) insufficient regeneration and scarring.[97] Macrophages and dendritic cells may play a role in the initiation as well as the progression of LN.[98 99]

It is, however, a question why immune complexes deposit in the kidney in the first place and what factors influence the location of these immune deposits. Traditionally, it was believed that immune complex deposition is a passive process. Now, several studies provide arguments against this notion. Yung *et al.* demonstrated binding of anti-dsDNA antibodies to mesangial annexin II. They also showed that this binding correlated with disease activity, and that annexin II colocalized with IgG and C3 deposits in human and murine LN.[100] In murine experimental LN Krishnan *et al.* demonstrated that only anti-DNA antibodies with glomerular basement membrane binding capacity were able to activate complement and induce proteinuria.[101] These studies suggest that cross-reactivities of anti-DNA antibodies may be responsible for initiating LN. In contrast, Mjelle *et al.* provide evidence that antibodies bind to nucleosomal antigens which in turn bind to components of mesangial matrix and the glomerular basement membrane.[102] Finally, failure to dismantle NETs has been shown to be correlated with kidney involvement in lupus, suggesting that neutrophils undergoing NETosis in the glomerulus may provide an additional source of nuclear antigens.[103]

Thesis outline

Diagnosing LN is the topic of the first two chapters. In **chapter 2** an investigation of the interobserver agreement in the recognition of class III and class IV LN nephritis is described. In **chapter 3** possible changes to be made to the current classification system of LN in order to further improve its usefulness and reproducibility are discussed.

In **chapter 4** of this thesis the comparison of six treatment guidelines of LN is presented, determining common ground in the treatment of LN between the guidelines and highlighting differences. These differences are areas where further research into the optimal treatment strategy is warranted.

The last three chapters of this thesis will focus on the pathogenesis of SLE, starting with the role of Mc. In the work described in **chapter 5** we investigated if Mc is more prevalent in peripheral blood of women with SLE than in controls. In the work described in **chapter 6** we aimed to determine if kinetics of Mc during and after pregnancy may be responsible for the difference we observed between SLE patients and controls. In order to gain further insight into the pathogenesis of LN, we compared patients with sporadic LN to patients with familial LN focusing on genetic, clinical, and histopathological aspects, as described in **chapter 7**.

Finally, in **chapter 8** we summarize and discuss the results of the research presented in the aforementioned chapters.

References

1. D'Cruz D. Systemic lupus erythematosus. *Lancet* 2007;369(9561):587-96.

2. Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. *Lupus* 2006;15(5):308-18.

3. Tucker LB, Menon S, Schaller JG*, et al.* Adult- and childhood-onset systemic lupus erythematosus: a comparison of onset, clinical features, serology, and outcome. *Br J Rheumatol* 1995;34(9):866-72.

4. Tucker LB, Uribe AG, Fernandez M*, et al.* Adolescent onset of lupus results in more aggressive disease and worse outcomes: results of a nested matched case-control study within LUMINA, a multiethnic US cohort (LUMINA LVII). *Lupus* 2008;17(4):314-22.

5. Seligman VA, Lum RF, Olson JL*, et al.* Demographic differences in the development of lupus nephritis: a retrospective analysis. *Am J Med* 2002;112(9):726-9.

6. Somers EC, Marder W, Cagnoli P*, et al.* Population-based incidence and prevalence of systemic lupus erythematosus: the Michigan Lupus Epidemiology and Surveillance program. *Arthritis Rheumatol* 2014;66(2):369-78.

7. Lim SS, Bayakly AR, Helmick CG*, et al.* The incidence and prevalence of systemic lupus erythematosus, 2002-2004: The Georgia Lupus Registry. *Arthritis Rheumatol* 2014;66(2):357-68.

8. Cohen AS, Reynolds WE, Franklin EC*, et al.* Preliminary criteria for the classification of systemic lupus erythematosus. *Bull Rheum Dis* 1971;21:643-48.

9. Tan EM, Cohen AS, Fries JF*, et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25(11):1271-7.

10. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40(9):1725.

11. Petri M, Orbai AM, Alarcon GS*, et al.* Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;64(8):2677-86.

12. Willicombe M, Moss J, Moran L*, et al.* Tubuloreticular Inclusions in Renal Allografts Associate with Viral Infections and Donor-Specific Antibodies. *J Am Soc Nephrol* 2016;27(7):2188-95.

13. Wen Y-K, Chen M-L. Clinicopathological study of originally non-lupus "full-house" nephropathy. *Ren Fail* 2010;32:1025-30.

14. Bajema IM, Bruijn JA. What stuff is this! A historical perspective on fibrinoid necrosis. *J Pathol* 2000;191(3):235-8.

15. Weening JJ, D'Agati VD, Schwartz MM*, et al.* The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol* 2004;15(2):241-50.

16. Weening JJ, D'Agati VD, Schwartz MM*, et al.* The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 2004;65(2):521-30.

17. Bertsias G, Ioannidis JP, Boletis J*, et al.* EULAR recommendations for the management of systemic lupus erythematosus. Report of a Task Force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics. *Ann Rheum Dis* 2008;67(2):195-205.

18. Austin HA, 3rd, Klippel JH, Balow JE*, et al.* Therapy of lupus nephritis. Controlled trial of prednisone and cytotoxic drugs. *N Engl J Med* 1986;314(10):614-9.

19. Boumpas DT, Austin HA, Balow JE*, et al.* Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. *Lancet* 1992;340(8822):741-45.

20. Gourley MF, Austin HA, 3rd, Scott D*, et al.* Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis. A randomized, controlled trial. *Ann Intern Med* 1996;125(7):549-57.

21. Appel GB, Contreras G, Dooley MA*, et al.* Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. *J Am Soc Nephrol* 2009;20(5):1103-12.

22. Murphy G, Lisnevskaia L, Isenberg D. Systemic lupus erythematosus and other autoimmune rheumatic diseases: challenges to treatment. *Lancet* 2013;382(9894):809-18.

23. Jordan N, D'Cruz D. Key issues in the management of patients with systemic lupus erythematosus: latest developments and clinical implications. *Ther Adv Musculoskelet Dis* 2015;7(6):234-46.

24. Navarra SV, Guzman RM, Gallacher AE*, et al.* Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 2011;377(9767):721-31.

25. Furie R, Petri M, Zamani O*, et al.* A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum* 2011;63(12):3918-30.

26. Block SR, Winfield JB, Lockshin MD*, et al.* Studies of twins with systemic lupus erythematosus. A review of the literature and presentation of 12 additional sets. *Am J Med* 1975;59(4):533-52.

27. Deapen D, Escalante A, Weinrib L*, et al.* A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum* 1992;35(3):311-8.

28. Alarcon-Segovia D, Alarcon-Riquelme ME, Cardiel MH*, et al.* Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort. *Arthritis Rheum* 2005;52(4):1138-47.

29. Yasutomo K, Horiuchi T, Kagami S*, et al.* Mutation of DNASE1 in people with systemic lupus erythematosus. *Nat Genet* 2001;28(4):313-4.

30. Han JW, Zheng HF, Cui Y*, et al.* Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat Genet* 2009;41(11):1234-7.

31. Yang W, Shen N, Ye DQ*, et al.* Genome-wide association study in Asian populations identifies variants in ETS1 and WDFY4 associated with systemic lupus erythematosus. *PLoS Genet* 2010;6(2):e1000841.

32. Armstrong DL, Zidovetzki R, Alarcon-Riquelme ME*, et al.* GWAS identifies novel SLE susceptibility genes and explains the association of the HLA region. *Genes Immun* 2014;15(6):347-54.

33. Graham RR, Cotsapas C, Davies L*, et al.* Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. *Nat Genet* 2008;40(9):1059-61.

34. Harley JB, Alarcon-Riquelme ME, Criswell LA*, et al.* Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat Genet* 2008;40(2):204-10.

35. Hom G, Graham RR, Modrek B*, et al.* Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. *N Engl J Med* 2008;358(9):900-9.

36. Okada Y, Shimane K, Kochi Y*, et al.* A genome-wide association study identified AFF1 as a susceptibility locus for systemic lupus eyrthematosus in Japanese. *PLoS Genet* 2012;8(1):e1002455.

37. Lee HS, Kim T, Bang SY*, et al.* Ethnic specificity of lupus-associated loci identified in a genome-wide association study in Korean women. *Ann Rheum Dis* 2014;73(6):1240-5.

38. Kozyrev SV, Abelson AK, Wojcik J*, et al.* Functional variants in the B-cell gene BANK1 are associated with systemic lupus erythematosus. *Nat Genet* 2008;40(2):211-6.

39. Tsokos GC. Systemic lupus erythematosus. *N Engl J Med* 2011;365:2110-21.

40. Konya C, Paz Z, Tsokos GC. The role of T cells in systemic lupus erythematosus: an update. *Curr Opin Rheumatol* 2014;26(5):493-501.

41. Bird AK, Meednu N, Anolik JH. New insights into B cell biology in systemic lupus erythematosus and Sjogren's syndrome. *Curr Opin Rheumatol* 2015;27(5):461-7.

42. Orme J, Mohan C. Macrophages and neutrophils in SLE-An online molecular catalog. *Autoimmun Rev* 2012;11(5):365-72.

43. Smith CK, Kaplan MJ. The role of neutrophils in the pathogenesis of systemic lupus erythematosus. *Curr Opin Rheumatol* 2015;27(5):448-53.

44. Rekvig OP, Van der Vlag J. The pathogenesis and diagnosis of systemic lupus erythematosus: still not resolved. *Semin Immunopathol* 2014;36(3):301-11.

45. Johnston I. The Iliad by Homer. Arlington, VA, USA: Richer Resources Publications, 2006.

46. Ford CE. Mosaics and chimaeras. *Br Med Bull* 1969;25(1):104-9.

47. Lagaaij EL, Cramer-Knijnenburg GF, van Kemenade FJ*, et al.* Endothelial cell chimerism after renal transplantation and vascular rejection. *Lancet* 2001;357(9249):33-37.

48. Korbling M, Katz RL, Khanna A*, et al.* Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med* 2002;346(10):738-46.

49. Lee TH, Paglieroni T, Ohto H*, et al.* Survival of donor leukocyte subpopulations in immunocompetent transfusion recipients: frequent long-term microchimerism in severe trauma patients. *Blood* 1999;93(9):3127-39.

50. Walknowska J, Conte FA, Grumbach MM. Practical and theoretical implications of fetal-maternal lymphocyte transfer. *Lancet* 1969;293(7606):1119-22.

51. Sato T, Fujimori K, Sato A*, et al.* Microchimerism after induced or spontaneous abortion. *Obstet Gynecol* 2008;112(3):593-97.

52. Peterson SE, Nelson JL, Guthrie KA*, et al.* Prospective assessment of fetal-maternal cell transfer in miscarriage and pregnancy termination. *Hum Reprod* 2012;27(9):2607-12.

53. Ariga H, Ohto H, Busch MP*, et al.* Kinetics of fetal cellular and cell-free DNA in the maternal circulation during and after pregnancy: implications for noninvasive prenatal diagnosis. *Transfusion* 2001;41(12):1524-30.

54. Hamada H, Arinami T, Hamaguchi H*, et al.* Fetal nucleated cells in maternal peripheral blood after delivery. *Am J Obstet Gynecol* 1994;170(4):1188-93.

55. Hamada H, Arinami T, Kubo T*, et al.* Fetal nucleated cells in maternal peripheral blood: frequency and relationship to gestational age. *Hum Genet* 1993;91(5):427-32.

56. Adams Waldorf K. Dynamic Changes in Fetal Microchimerism in Maternal Peripheral Blood Mononuclear Cells, CD4+ and CD8+ cells in Normal Pregnancy. *Placenta* 2010;31(7):589-94.

57. Hsieh TT, Pao CC, Hor JJ*, et al.* Presence of fetal cells in maternal circulation after delivery. *Hum Genet* 1993;92(2):204-05.

58. Mersy E, Smits LJ, van Winden LA*, et al.* Noninvasive detection of fetal trisomy 21: systematic review and report of quality and outcomes of diagnostic accuracy studies performed between 1997 and 2012. *Hum Reprod Update* 2013;19(4):318-29.

59. Bianchi DW, Zickwolf GK, Weil GJ*, et al.* Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci U S A* 1996;93(2):705-08.

60. Maloney S, Smith A, Furst DE*, et al.* Microchimerism of maternal origin persists into adult life. *J Clin Invest* 1999;104(1):41-47.

61. Schechter GP, Whang-Peng J, McFarland W. Circulation of donor lymphocytes after blood transfusion in man. *Blood* 1977;49(4):651-6.

62. Adams PT, Davenport RD, Reardon DA*, et al.* Detection of circulating donor white blood cells in patients receiving multiple transfusions. *Blood* 1992;80(2):551-5.

63. Dunne JR, Lee TH, Burns C*, et al.* Transfusion-associated microchimerism in combat casualties. *J Trauma* 2008;64(2 Suppl):S92-7; discussion S97-8.

64. Utter GH, Owings JT, Lee TH*, et al.* Blood transfusion is associated with donor leukocyte microchimerism in trauma patients. *J Trauma* 2004;57(4):702-7.

65. Kruskall MS, Lee TH, Assmann SF*, et al.* Survival of transfused donor white blood cells in HIV-infected recipients. *Blood* 2001;98(2):272-9.

66. Reed W, Lee TH, Vichinsky EP*, et al.* Sample suitability for the detection of minor white cell populations (microchimerism) by polymerase chain reaction. *Transfusion* 1998;38(11-12):1041-5.

67. Bloch EM, Busch MP, Lee TH*, et al.* Microchimerism in the transfused obstetric population. *Vox Sang* 2014;107(4):428-30.

68. Elwood ET, Larsen CP, Maurer DH*, et al.* Microchimerism and rejection in clinical transplantation. *Lancet* 1997;349(9062):1358-60.

69. Dutta P, Burlingham WJ. Microchimerism: tolerance vs. sensitization. *Curr Opin Organ Transplant* 2011;16(4):359-65.

70. Curcio M, Cantarovich D, Barbuti S*, et al.* Association of donor-specific microchimerism with graft dysfunction in kidney transplant patients. *Transpl Immunol* 2012;26(2-3):151-5.

71. Koopmans M, Kremer Hovinga I, Baelde HJ*, et al.* Chimerism in kidneys, livers and hearts of normal women: implications for transplantation studies. *Am J Transplant* 2005;5(6):1495-502.

72. Pujal JM, Gallardo D. PCR-based methodology for molecular microchimerism detection and quantification. *Exp Biol Med* 2008;233(9):1161-70.

73. Alizadeh M, Bernard M, Danic B*, et al.* Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. *Blood* 2002;99:4618-25.

74. Jimenez-Velasco A, Barrios M, Roman-Gomez J*, et al.* Reliable quantification of hematopoietic chimerism after allogeneic transplantation for acute leukemia using amplification by real-time PCR of null alleles and insertion/deletion polymorphisms. *Leukemia* 2005;19(3):336-43.

75. Maas F, Schaap N, Kolen S*, et al.* Quantification of donor and recipient hemopoietic cells by real-time PCR of single nucleotide polymorphisms. *Leukemia* 2003;17(3):621-29.

76. Schmorl G. *Pathologisch-anatomische Untersuchungen ueber Puerperal Eklampsie*. Leipzig: Vogel, 1896.

77. Buurma AJ, Penning ME, Prins F*, et al.* Preeclampsia is associated with the presence of transcriptionally active placental fragments in the maternal lung. *Hypertension* 2013;62(3):608-13.

78. Rijnink EC, Penning ME, Wolterbeek R*, et al.* Tissue microchimerism is increased during pregnancy: a human autopsy study. *Mol Hum Reprod* 2015;21(11):857-64.

79. Mold JE, Michaelsson J, Burt TD*, et al.* Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. *Science* 2008;322(5907):1562-5.

80. Lisnevskaia L, Murphy G, Isenberg D. Systemic lupus erythematosus. *Lancet* 2014;384(9957):1878-88.

81. Via CS, Shearer GM. T-cell interactions in autoimmunity: insights from a murine model of graft-versus-host disease. *Immunol Today* 1988;9(7-8):207-13.

82. Gleichmann E, Van Elven EH, Van der Veen JP. A systemic lupus erythematosus (SLE)-like disease in mice induced by abnormal T-B cell cooperation. Preferential formation of autoantibodies characteristic of SLE. *Eur J Immunol* 1982;12(2):152-9.

83. Kremer Hovinga I, Koopmans M, Baelde HJ*, et al.* Chimerism occurs twice as often in lupus nephritis as in normal kidneys. *Arthritis Rheum* 2006;54(9):2944-50.

84. Kremer Hovinga I, Koopmans M, Baelde HJ*, et al.* Tissue chimerism in systemic lupus erythematosus is related to injury. *Ann Rheum Dis* 2007;66(12):1568-73.

85. Florim GM, Caldas HC, de Melo JC*, et al.* Fetal microchimerism in kidney biopsies of lupus nephritis patients may be associated with a beneficial effect. *Arthritis Res Ther* 2015;17(1):101.

86. Abbud Filho M, Pavarino-Bertelli EC, Alvarenga MP*, et al.* Systemic lupus erythematosus and microchimerism in autoimmunity. *Transplant Proc* 2002;34:2951-52.

87. Kekow M, Barleben M, Drynda S*, et al.* Long-term persistence and effects of fetal microchimerisms on disease onset and status in a cohort of women with rheumatoid arthritis and systemic lupus erythematosus. *BMC Musculoskelet Disord* 2013;14:325.

88. Gannage M, Amoura Z, Lantz O*, et al.* Feto-maternal microchimerism in connective tissue diseases. *Eur J Immunol* 2002;32(12):3405-13.

89. Mosca M, Curcio M, Lapi S*, et al.* Correlations of Y chromosome microchimerism with disease activity in patients with SLE: analysis of preliminary data. *Ann Rheum Dis* 2003;62(7):651-4.

90. Kanold AMJ, Svenungsson E, Gunnarsson I*, et al.* A Research Study of the Association between Maternal Microchimerism and Systemic Lupus Erythematosus in Adults: A Comparison between Patients and Healthy Controls Based on Single-Nucleotide Polymorphism Using Quantitative Real-Time PCR. *PLoS One* 2013;8(9):e74534.

91. Kremer Hovinga I, Koopmans M, de Heer E*, et al.* Chimerism in systemic lupus erythematosus--three hypotheses. *Rheumatology (Oxford)* 2007;46(2):200-08.

92. van Kampen CA, Versteeg-vd Voort Maarschalk MF, Langerak-Langerak J*, et al.* Kinetics of the pregnancy-induced humoral and cellular immune response against the paternal HLA class I antigens of the child. *Hum Immunol* 2002;63(6):452-8.

93. Seppanen E, Fisk NM, Khosrotehrani K. Pregnancy-acquired fetal progenitor cells. *J Reprod Immunol* 2013;97(1):27-35.

94. O'Donoghue K, Chan J, de la Fuente J*, et al.* Microchimerism in female bone marrow and bone decades after fetal mesenchymal stem-cell trafficking in pregnancy. *Lancet* 2004;364(9429):179-82.

95. Nassar D, Droitcourt C, Mathieu-d'Argent E*, et al.* Fetal progenitor cells naturally transferred through pregnancy participate in inflammation and angiogenesis during wound healing. *FASEB J* 2012;26(1):149-57.

96. Khosrotehrani K, Reyes RR, Johnson KL*, et al.* Fetal cells participate over time in the response to specific types of murine maternal hepatic injury. *Hum Reprod* 2007;22(3):654-61.

97. Lorenz G, Desai J, Anders HJ. Lupus nephritis: update on mechanisms of systemic autoimmunity and kidney immunopathology. *Curr Opin Nephrol Hypertens* 2014;23(3):211-7.

98. Rogers NM, Ferenbach DA, Isenberg JS*, et al.* Dendritic cells and macrophages in the kidney: a spectrum of good and evil. *Nat Rev Nephrol* 2014;10(11):625-43.

99. Davidson A, Bethunaickan R, Berthier C*, et al.* Molecular studies of lupus nephritis kidneys. *Immunol Res* 2015;63(1-3):187-96.

100. Yung S, Cheung KF, Zhang Q*, et al.* Anti-dsDNA antibodies bind to mesangial annexin II in lupus nephritis. *J Am Soc Nephrol* 2010;21(11):1912-27.

101. Krishnan MR, Wang C, Marion TN. Anti-DNA autoantibodies initiate experimental lupus nephritis by binding directly to the glomerular basement membrane in mice. *Kidney Int* 2012;82(2):184-92.

102. Mjelle JE, Rekvig OP, Van Der Vlag J*, et al.* Nephritogenic antibodies bind in glomeruli through interaction with exposed chromatin fragments and not with renal cross-reactive antigens. *Autoimmunity* 2011;44(5):373-83.

103. Hakkim A, Furnrohr BG, Amann K*, et al.* Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc Natl Acad Sci U S A* 2010;107(21):9813-8.