ation of cytokines which prime the neutophils to express ANCA antigens on their surface. The binding of ANCA induces neutrophil activation with the release of hydrolytic enzymes and toxic oxygen metabolites. Subsequent endothelial cell death, which probably involves both apoptosis and lytic necrosis, occurs if the neutrophils were previously bound to the endothelial cells. This is enabled via cytokine-induced cell adhesion molecules expressed on endothelial cells and occurs particularly at sites where leukocytes are in close contact with vessel walls, as in kidney glomerulus and in pulmonary alveolar capillaries. Furthermore, it has been shown that highly cationic ANCA antigens that are released from activated neutrophils may bind to the negatively charged endothelial cells and extracellular matrix structures, such as the glomerular basement membrane. There they can act as planted antigens for *in situ* immune complex formation and local complement activation may augment the tissue injury. Moreover, some studies suggest that endothelial cells, if influenced by cytokines, produce and express PR3 on their surface and in this case ANCA could act as antiendothelial cell antibodies and kill endothelial cells by an antibody-dependent cytotoxicity. It has also been hypothesized that bound PR3 can be recognized by specific T lymphocytes.

There are an increasing number of experimental animal models that simulate ANCA-positive human disease at least in some features, although none of them is an ideal model for pauciimmune ANCA-positive vasculitis in humans.

References

- Drool K, Gross WL. *Wegeners granulomatosis: Disease course, assessment of activity and extent and treatment.* Lupus 1998; 7: 258-91.
- Jennette Jc, Falk RJ. *Antineutrophil cytoplasmic autoantibodies - associated diseases - a pathologist' perspective.* Am J Kid Dis 1991; 18: 164-170.
- Jennette C, Eslk RJ, Andrsssy K *et* sl. *Nomenclature of systemic vasculitides. Proposal of an International Consensus Conference.* Arthritis Rheum 1994; 37: 187-192.
- *Jennette C,* Falk RJ. *Pathogenesis of the vascular and glomerular damage in At'JCA-positive vasculitis.* Nephrol Disi Trsnplsnt 1998; 13(Suppl. 1): 16-20.
- Ksllenberg 0GM, Brouwer E, Weening JJ St sl. *Anti-neutrophil cytoplasmic antibodies: Current diagnostic and pathophysiological potential.* Kidney *Int* 1994; 46: 1-15.
- Lessvre P. *The diagnostic and prognostic significance of ANCA.* Ren Fail 1996; 18: 803-812.
- Lhote F, cohen P, Guillevin L. *Polyarteritis nodosa, microscopic polyangiitis and Churg-Strauss syndrome.* Lupus 1996; 7: 238-258.

949–Y–,~ – –

Vascular pathology in systemic lupus erythematosus: Crossroads of immune complex vasculitis and vasculopathy, thrombotic microangiopathy and arteriosclerosis

D. Ferluga

Institute of Pathology, Faculty of Medicine, University of Ljubljana, Slovenia.

Systemic lupus erythematosus (SLE) is a prototype of a multisystem autoimmune connective tissue disease, marked by immune complex-mediated lesions of blood vessels in diverse organs. It is a disease with a myriad of diverse clinical manifestations and a fairly unpredictable exacerbating course, which is influenced by the current therapeutic approaches with corticosteroids and immunosuppressive drugs. Despite the growing understanding of the cellular and molecular events, the obviously very complex etiopathogenesis of SLE still remains unsolved. It appears that the interplay of etiologic factors, genetic, hormonal and environmental, results in a failure of the immune regulatory mechanisms characterized by a T-cell dysfunction, a B-cell activation and an imbalance in the production of cytokines. A dominant consequent feature is an overproduction of a bewildering array of autoantibodies, of which over 50 are currently well characterized, particularly those against diverse nuclear and cytoplasmic components of the cell. Three basic pathogenetic mechanisms have been suggested; i) injury related to deposition of circulating and *in* situ formed immune complexes, double-stranded DNA (dsDNA) – anti dsDNA being of particular significance; ii) the direct cytotoxic effect of autoantibodies; and iii) functional effect of autoantibodies, such as autoantibodies to phospholipids involved in thrombogenesis, anti-ribonucleoprotein (RNP) and anti-DNA penetrating into live cells may cause apoptosis and thus stimulate antigen-driven production of anti-DNA'nucleosome autoantibodies.

Lupus vasculopathy and vasculitis in diverse organs

Vascular involvement characterizes the pathology of diverse organs in SLE and seems to be crucial for the majority of clinical manifestations. Widespread vascular lesions were described in the most frequently cited classical study of SLE pathology by Klemperer *et* al. in 1941. They reported microvascular injury with "fibrinoid" degeneration and necrosis of predominantly small arteries, arterioles and capillaries. Significantly less frequent inflammatory or thrombotic effects of diverse vessels, including even large arteries and veins, have not inspired systematic investigations until recently.

Our experience is based on a systematic study of 342 kidney biopsy specimens in 266 SLE patients, 131 skin biopsy samples in 114 patients, 125 biopsy samples of skeletal muscle in 112 patients and on the tissue samples of diverse organs obtained from autopsies of 37 patients. The most consistent and characteristic were immunofluorescence microscopic findings of granular deposits of immunoglobulins, predominantly lgG, and complement components, especially Clq, in kidney glomeruli (99%), choroid plexus (82%), skin (75%), spleen (73%), heart (71%), salivary glands (57%), brain (56%), lung (49%), liver (42%) and skeletal muscle (36%). The deposition of immune reactants in all organs, with the choroid plexus as the only clear-cut exception, correlated with non inflammatory vasculopathy and inflammatory vascular and extravascular changes. Since the pathology of the kidney in SLE has been studied by far the most extensively and systematically, the following text will be devoted to renal vascular lesions.

Luous glomerulaneohritis

The glomeruli are by far the most commonly affected by deposits of wide arrays of circulating and *in situ* formed immune complexes in SLE. Glomerular capillaritis results in different types of lupus glomerulonephritis. In our large series of 379 kidney biopsies and autopsy samples, the following glomerular distribution patterns were found with the following incidence; nil (1.4%), mesangial (15.5%), mesangial-subendothelial (10.6%), subepithelial (4.4%), mesangial-subepithelial (12.0%) and, the most frequent and characteristic, mesangial-transmembranous (56.1%). A clear-cut posi-

tive correlation was demonstrated between the extent, composition and distribution pattern ot the glomerular immune deposits and the intensity and pattern of the inflammatory reaction. Practically all histomorphological forms of glomerulonephritis can be observed in SLE, including as the most characteristic, mixed membranous and proliferative glomerulonephritis. According to the widely accepted World Health Organization (WHO) classification, the following incidence of various classes was observed in our series: class I, no glomerular changes (4.0%); class II, mesangiopathy (11.6%); class III, focal glomerulonephritis (24.0%); class IV, diffuse proliferative glomerulonephritis **(47.2%);** class V, diffuse membranous glomerulonephritis (11.9%) and class VI, terminal sclerosing glomerulonephritis (1.3%).

Renal extraglomerular vascular pathology in SLE

In their classical description of the autopsy pathology in SLE patients, Klemperer ef *al.* (1941) pointed out the characteristic subendothelial imbibition ot small arteries and arterioles by fibrinoid material, particularly in the kidneys. In subsequent decades, with systemic use of renal biopsy, extraglomerular vascular pathology investigation was overshadowed by comprehensive studies of a heterogeneous array of glomerular changes widely accepted as fundamental for the classification of lupus nephritis. Only a limited number of systematic studies in this field have been published so far and there is still no internationally approved consensus about the classification of lupus vascular changes and their prognostic significance. In the 1980s, histomorphological vascular changes in SLE were studied by a few investigators using special techniques on renal biopsy material. The term lupus microangiopathy was introduced and although monotonous and basically non-inflammatory, the histopathology was suggested to be causally related to local immune deposits. Only recently have additional histomorphological patterns of extraglomerular vascular injury in SLE patients been established and ascribed to diverse pathogenetic mechanisms. Our results are presented following roughly the approach tor classification introduced by Appel ef *al.* in 1994.

Immune deposit-associated lucius vasculopathy and vasculitis

Granular deposits of immune reactants, characterized by a predominance of IgG and Clq, within the intima, media and/or adventitia of arterioles and small arteries, rarely within venules, were found by immunofluorescence and electron microscopy in the renal biopsies of 153 (56.4%) out of 261 SLE patients. Associated vascular changes observed by traditional light microscopy could be described as noninflammatory (lupus vasculopathy 107/266 (40.2%)) or inflammatory (lupus vasculitis 43/266 (16.2%)).

Uncomplicated vascular immune deposits

The normal appearance of extraglomerular renal vessels was found by all techniques in 70 patients (26.3%), predominantly those showing mild and/or inactive torms of lupus nephritis. Despite the presence of vascular granular immune deposits, blood vessels looked uninvolved by light microscopy in 37 (13.9%), and in 19 (7.1%) an unremarkable thickening of the small blood vessels was observed. The association of arteriosclerotic changes with granular vascular immune deposits was demonstrated in 32 SLE patients (12.0%).

Hyalinizina luPus microangiociathy

The most typical vascular lesion in SLE deserves today a more precise detinition and delineation from other histomorphologically similar conditions. Hyalinizing lupus microangiopathy, also known as lupus vasculopathy, noninflammatory lupus microangiopathy and angiitis in SLE, is characterized by an imbibition of the walls of renal arterioles and small inferlobular arteries, particularly their intima, by a homogeneous, eosinophilic material, which occasionally extends into the media and protrudes or forms precipitates in the vascular lumina. Immunofluorescence microscopy has shown that this hyaline material contains immune reactants, predominantly lgG and Clq, and rarely traces of fibrin/fibrinogen. Occasionally, structured vascular immune deposits may show characteristic "fingerprint" figures by electron microscopy. Nonimmune complex-related arteriolohyalinosis can be distinguished by its frequently nodular pattern and the predominance of 1gM and C3. Hyalinizing lupus microangiopathy may share similarities with thrombotic microangiopathy, which is characterized by fibrin thrombi and by the abundance of fibrin/fibrinogen within the subendothelial insudate.

Pure hyalinizing lupus microangiopathy was observed in 19 patients (7.1%) in our series whereas in an additional 16 (6.0%) it was found accompanied by an inflammatory reaction, usually mild. Investigators have noted superimposed changes from scattered endothelial and smooth muscle cell necrosis to frank fibrinoid necrosis of the vessel walls, the latter being considered by Appel et *al.* (1994) an acceptable reason for the establishment of a separate category termed non inflammatory necrotizing vasculopathy. The hypothesis has been put forward that it may represent a complication of a more severe form of vascular immune deposition resembling focal segmental necrotfzing lupus glomerulonephritis (class III according to the WHO classification) but other recently described mechanisms, including antiendothelial cell antibodies and T-cell mediated immunological injury, have also been considered.

Immune deposit-associated lucius vasculitis

This category of SLE-related vascular pathology has not been unanimously defined nor has the diagnostic criteria so far been unified. The significant differences in the prevalence of lupus vasculitis reported b y different research groups are therefore not surprising. Despite the rarity or even absence of leukocytic infiltration, hyalinizing vasculopathy was declared lupus vasculitis by some investigators due to a coincidence of necrotizing changes. In contrast, following a very strict definition of lupus vasculitis, which as a prerequisite includes inflammatory cell infiltrates in the arterial media, not a single case of renal vasculitis was verified in a large series of SLE patients. Others, who consider as true inflammatory only those vascular changes in SLE patients that resemble p01-yarteritis nodosa, reported the incidence rates of renal lupus vasculitis to be 0.3-2.4%.

We included in this subgroup all patients whose kidney biopsies showed, in addition to local granular immune deposits, necrotizing, cell infiltrative and/or proliferative changes in the vessel walls, irrespective of their intensity. Applying such a broad definition, the prevalence of renal lupus vasculitis in our series was 16.2%. Of 43 patients, 16 (6.0%) expressed histologically a combination of a full-blown picture of hyalinizing lupus vasculopathy. Various inflammatory changes were mild in the majority of patients but one patient who fulfilled the American Rheumatism Association's criteria for SLE, including positive anti-DNA during the course of the disease, showed at autopsy a histopathology of poi-

yarteritis nodosa and her serum, taken before death, was found to be positive for perinuclear antineutrophil cytoplasmic antibodies. A similar recently published observation has been interpreted as a transformation of SLE into polyarteritis nodosa.

Immune complexes can localize in vessel walls by deposition from the circulation, by *in situ* binding of the circulating antibody to previously planted antigen or by both mechanisms. Deposited immune complexes trigger vascular injury and inflammatory response by activation of humoral and cellular mediator systems. The humoral immune system involves protease cascades, including the activation of complement with a particularly important release of anaphylatoxins and chemoattractants, 03a and C5a, and eventually a generation of a cytotoxic membrane attack complex C5b-9. Activation of leukocytes as well as of endothelial cells promotes adhesion between these cells, penetration of leukocytes into vessel walls and release of injurious leukocyte products, such as lytic enzymes and toxic oxygen metabolites. A number of questions, regarding immune complexes vascular precipitation and their role in the mediation of tissue injury and inflammation remain to be elucidated.

Thrombotic microanolovathy in .SLE

Glomerular hyaline capillary occlusive thrombi rather frequently occurring in patients with severe forms of lupus nephritis usually represent huge protruding subendothelial deposits or intraluminal precipitates of immune complexes. Nevertheless, additional tentative pathogenic mechanisms have to be considered. Serious exacerbations of SLE can be complicated by a failure of multiple organs due to leukoocclusive microangiopathy, which is best modeled by the Schwartzman phenomenon. It has been hypothesized that the exuberant systemic release of anaphylatoxins and/or cytokines may cause widespread activation of the inflammatory cells as well as adherence of platelets and neutrophils to the activated vascular endothelium.

The endothelium appears to be a key target in the pathogenesis of vasculitides and atherosclerosis. Antiendothelial cell antibodies have been described in sera from patients with a wide variety of connective tissue disorders, including SLE. Nevertheless, their exact antigenic specificities remain unknown and their tentative cytotoxicity has not yet been satisfactorily proven.

A heterogeneous family of antiphospholipid autoantibodies, termed anticardiolipin antibodies or lupus anticoagulant, according to the testing methodology, reveals a convincing association with occlusive thrombotic vascular histopathology and antiphospholipid syndrome, clinically characterized by multiple thromboembolic events, thrombocytopenia and recurrent fetal loss. Proposed mechanisms include direct endothelial cell injury, antibody-mediated platelet activation and inhibition of endogenous anticoagulants. It is known that about a third of SLE patients have antiphospholipid antibodies and that about 15% fulfill the diagnostic criteria for secondary antiphospholipid syndrome. In our series of 14 consecutive autopsied SLE patients, anticardiolipin antibodies were considered positive in 13, which may suggest their contribution to the fatal course of the disease. Specific thrombotic changes detected in eight patients at different stages affected mainly the small blood vessels and in only three patients did it affect the larger arteries and veins. Both thrombotic and thrombotic microangiopathy compatible changes were significantly more common in the medium to high anticardiolipin antibody positive SLE patients. Histological changes characterizing acute or chronic glomerular and/or vascular thrombotic microangiopathy were detected in our series of 266 SLE patients in 11(4.1%) in the kidney biopsies, usually accompanied by immune deposit-associated histopathology and, fairly frequently, arteriosclerosis. Diagnostically significant findings of subendothelial plasma insudation containing 1gM and fibrin, fibrin thrombi and mucoid intimal fibrosis of arteries are in accordance with the hypothesis of the endothehal cell as a key target.

Premature arteriosclerosis in SLE

Over 25% of late deaths of SLE patients can clinically be attributed to the atherosclerotic process. Over 50% of autopsied SLE patients demonstrated moderate to severe generalized atherosclerosis. Several studies have suggested an association between SLE and premature atherosclerosis. Arteriosclerotic changes were demonstrated in the kidney biopsies of 101 (38.0%) out of 266 SLE patients of our series: arteriolosclerosis in 16.9%, arteriolohyalinosis incorporating occasionally extracellular lipids in 14.3%, nflmal arterial fibrosis and fibroelastosis in 31.2% and intrarenal atherosclerosis, never observed as a natural aging process, in 1.5%. The etiology of arterioloscierosis, much like that of SLE, is multifactorial. A number of proatherogenic risk factors in SLE patients have been established, such as dyslipidemias, corticosteroids, nephrotic syndrome and renal failure, hypertension and infections as well as immunological factors, including antiphosphohipid antibodies. Endothelial cell activation and injury, thrombogenesis, influx of plasma constituents and monocytes, proliferation of transformed smooth muscle cells and overproduction of the extracellular matrix are important events which may be shared in the pathogenesis of vasculitis, thrombotic microangiopathy and premature arteriosclerosis.

References

- Appel GB, Pirani CL, DAgati **V.** *Renal vascular complications of systemic lupus erythematosus.* Am J Soc Nephrol 1994; 4:1499-1515.
  Belmont HM, Abramson SB, Lie JT. *Pathology and pathogenesis of vascular injury in systemic lupus erythematosus.* Arthritis Rheum 1996; 39: 9-22.
- Bhathena OB, Sobel BJ, Migdal SD. *Noniflammatoiy renal microangiopathy of systemic lupus erythematosus ('Lupus Vasculitis').* Am J Nephrol 1981; 1: 144-159.
  Bridoux F, \Irtovsnik F, Noel C *et al. Renal thrombotic microangiopathy in systemic lupus erythematosus: Clinical correlations and long-term survival.* Nephrol Dial Transplant 1998; 13: 298-304.
- Cruz DO. *Vasculitis in systemic lupus eiythematosus.* Lupus 1998; 7: 270-274.
- George J, Schoenfeld Y. *Editorial. The anti-phospholipid (Hughes) syndrome: A crossroads of autoimmunity and atherosclerosis.* Lupus 1997; 6: 559-560.
- Jennette JC, Falk RJ. *Update on the pathobiology of vasculitis.* In: Schoen FJ, Gimbrone MA. (Eds.). Cardiovascular Pathology Clinicopathologic Correlations and pathogenetic mechanisms. Williams & Williams, Baltimore 1995; 156-172.
- ilizjak A, Perkovic T, Rozman B et al. *Skeletal muscle immune deposits in systemic lupus eiythematosus.* Scand J Rheumatol 1998; 27: 207-214.