The effect of cell death in the initiation of lupus nephritis

By

Kristin Fenton[1]

[1]Molecular Pathology Research Group, Institute of Medical Biology, Faculty of Health Sciences, University of Tromsø, N-9037Tromsø, Norway.

Corresponding author: Kristin A. Fenton. Molecular Pathology Research Group, Institute of Medical Biology, Faculty of Health Sciences, University of Tromsø, N-9037 Tromsø, Norway. Tlf: +4777646834, Fax: +4777645350, E-mail: kristin.fenton@uit.no

Abstract

Cell death and the release of chromatin have been demonstrated to activate the immune system producing autoantibodies against nuclear antigens in patients with systemic lupus erythematosus (SLE). Apoptosis, necrosis, necroptosis, secondary necrosis, autophagy and the clearance of dying cells by phagocytosis are processes believed to have a role in tolerance avoidance, activation of autoimmune lymphocytes and tissue damage by effector cells. The released chromatin not only activates the immune system. It also acts as antigen for the produced autoantibodies including anti-dsDNA antibodies. The subsequent immune complex formation is deposited within the basement membranes and the mesangial matrix of glomeruli. This may be considered as an initiating event in lupus nephritis. The origin of the released chromatin is still debated and here we discuss the possible mechanisms and cell sources.

Introduction

Chromatin or nucleosomes are the driving antigens in the induction of anti-double-stranded (ds)DNA antibodies. The presence of anti-dsDNA antibodies is a hallmark of Systemic lupus erythematosus (SLE). The process is driven by special, autoimmune T helper (Th) cells specific to epitopes in various DNA-binding nucleoproteins such as histones [1;2]. Anti-dsDNA antibodies form immune complexes with nucleosomes that deposits within basement membranes in the body, eg skin and kidney, and may cause a systemic inflammation [3-6]. Lupus nephritis is characterized by the deposition of such immune complexes within the mesangial matrix and basement membranes of glomeruli, in addition to deposition within the basal membrane of the main renal arteries and micro-capillaries surrounding the tubuli [7]. The binding of anti-dsDNA/nucleosomes immune complexes to basement membranes is mediated via nucleosomes. Nucleosomes show an affinity towards membrane components [8;9]. We have previously demonstrated that the production of anti-dsDNA abs, formation of immune complexes and subsequent deposition precedes presence of infiltrating immune cells within the kidneys and the development of proteinuria of lupus prone mice [10]. The release of nucleosomal antigens may therefore play a crucial role in the initiation of lupus nephritis.

Nucleosomes are complexes composed of dsDNA and histones. One nucleosome is composed of 180 basepairs of dsDNA and histone proteins organized as a protein octamer with the dsDNA wrapped in 1.65 turns of a superhelix [11]. An exterior linker histone stabilizes the structure together with a linker dsDNA connecting adjacent nucleosomes. Chromatin or nucleosomes contain protein complexes of DNA and histone binding proteins [12] and is normally located in the nucleus of the cell. Chromatin can be released during cell damage or death. However, mechanism like cell activation has also been shown to release nucleosomes in form of micro particles. The presence of nucleosomes has been detected in sera from normal individual and in patients with SLE [13]. In mice the levels of circulating nucleosomes decreases when anti-dsDNA antibody production increases during the progression of the disease, and may reflect formation and deposition of immune complexes [13]. Treatment with heparin prevented the deposition of immune complexes probably by making the nucleosomes more accessible for degradation by nucleases [14]. The main source of nucleosomes in SLE is believed to arrive from

dead cells of a apoptotic or necrotic origin. Possible mechanisms and cell sources of extracellular nucleosomes are discussed.

Mechanisms of programmed cell death as the origin for release of nucleosomes and activation of autoimmune cells

Apoptosis, or programmed cell death (PCD) is essential for embryonic development and renewal of tissue by eliminating cells that are abnormal and potentially dangerous [15]. PCD maintains homeostasis of the immune system, eg. after massive expansion of reactive T cells and B cells in response to infection [16]. This is important in order to sustain immune tolerance and is a key process in the positive and negative selection of B and T lymphocytes eliminating potential self-reactive cells [17]. Apoptosis can be initiated by several death receptors on the cell surface or from signals within the cell in response to stress, DNA damage, defective cells cycle etc [15]. Apoptosis is characterized by activation of caspases, DNA fragmentation and membrane blebb formation [18]. Importantly, apoptosis does not normally activate the immune system, and apoptotic cells are cleared by phagocytes without the release of nucleosomes and with little consequent inflammation. However, nucleosomes are exposed at the cell surface of apoptotic blebs [19]. Inefficient clearance of dying cells may result in the accumulation of apoptotic cell remnants leading to a process called secondary necrosis [20]. This will lead to degeneration of the cell remnants and release of nucleosomes and other damage-associated molecular patterns (DAMPs) like HMGB1, IL-1a, uric acid, mitochondrial content, and ATP like those observed during necrosis or necroptosis (see below)[21]. Incomplete chromatin digestion by phagocytes may also lead to the release of particles containing nucleic acids [20]. Chromatin modifications such as acetylation and methylation of histones have been demonstrated during apoptosis (reviewed in [22]) and autoantibodies specific for acetylated and methylated histone epitopes have been found in SLE patients and in experimental mice [23;24]. Since the apoptotic process may modify the nucleic acids to look more like foreign antigen e.g. a viral particle, the antigen presenting cells (APCs, dendritic cells and B cells) may take it up, recognize it as foreign and get activated [25]. Apoptotic blebs with chromatin modifications have been demonstrated to activate dendritic cells with subsequent IL-17 production of co-cultured T cells [26].

In contrast to apoptosis, necrotic cell death is characterized by cellular organ and cytoplasma swelling which leads to rapture of the plasma membrane and subsequent cell lysis [27]. It was originally considered as a non-controlled death mechanism, but recent data has pointed at certain tightly controlled types of necrosis called necroptosis [28]. This process is dependent on the serine-threonine kinase RIP1 (RIPK1) and the RIP1 family member RIP3 has been identified as a key mediator of caspase-independent cell death [29]. The interplay between apoptosis and necroptosis is important for the elimination of excess T cells after an immune response [30]. Interestingly, the pro inflammatory cytokine tumor necrosis factor (TNF) can induce apoptosis in certain cell types and necrosis on others [31]. Many other signals of the immune system like ligation of Fas, engagement of toll like receptors (TLR) or TCR by dsDNA and anti-CD3 antibodies respectively, have been shown to trigger necroptosis [32]. The induction of necrosis or necroptosis by pro-inflammatory cytokines and other triggers may increase the release of nucleosomes. Necroptosis has not been investigated in SLE but extensive studies on tumour necrosis factor-like weak inducer of apoptosis (TWEAK) indicate a central role for the TNF pathways in the induction of lupus nephritis [33].

Authophagy is a third mechanism that could lead to PCD.Autophagy is an intra-cellular degradation system that allows the cells to consume itself for energy and is triggered by stress conditions like starvation or could be a part of normal turnover of cellular contents [34]. Autophagy has been shown to be involved in the elimination of microorganisms, control of pro-inflammatory signaling, adaptive immunity and secretion of immune mediators (reviewed in [35]). Autophagy can rescue cells from the apoptotic process by interfering with the apoptotic signaling cascade. Inflammasome activation and secretion of immune mediators are regulated by autophagy through regulatory interactions with innate immune signaling pathways [35]. Autophagy has been demonstrated to be important in both T and B cell immunity by contributing to antigen presentation, T cell homeostasis and polarization, and to survival of mature B cells and required for plasmablast formation [35]. The impact of autophagy in SLE has recently been reviewed and shown to be activated [36]. Autophagy is increased in SLE patients and lupus prone mice [36]. It is involved in the survival of autoreactive B cells and required for the differentiation of plasmablasts [37]. An increased amount of autophagic compartments was observed in T cells from lupus prone mice and SLE patents, indicating a deregulation of autophagy in SLE [38].

In response to pathogens neutrophils may die in a process called NETosis [39]. Upon stimulation or engagement with pathogens neutrophils release extracellular traps (NETs) that consist of nucleosomes and neutrophil proteins. These may surround the pathogen and increase the likelihood of phagocytosis by macrophages [40]. NETs released by dying neutrophils are normally degraded by Dnase1 within the circulation [40]. Impaired degradation of NETs has been seen in sera from SLE patients and this was associated with lupus nephritis [41]. Recently published studies demonstrate that NETs can induce innate pDC activation through TLR9 and TLR7 [42;43]. Increased infiltration and activation of neutrophils within the kidney with subsequent release of NETs may be harmful in SLE. The NETs may be targeted by anti-dsDNA antibodies which may promote IC deposition within the mesangial matrix and GBM, therefore being a potentially crucial factor in development of lupus nephritis (see below).

Dying cells as source of released chromatin in lupus nephritis

Whether apoptotic kidney cells are the source of released renal chromatin is controversial. Some groups demonstrate increased apoptosis of residential kidneys cells [3;4;44;45], while others claim that there is no increase in apoptotic kidneys cells of lupus patients and in mouse models of lupus nephritis [46;47]. Looking at immune complex deposition there are several possible kidney cell types and infiltrating immune cells that may be a possible source of chromatin.

Glomerular mesangial cells, endothelial cells, and podocytes

The first deposition of immune complexes is observed within the glomerular mesangial matrix. It is therefore likely that the source of released chromatin may come from dying mesangial cells. One of the theories of anti-dsDNA ab deposition is the "planted antigen" theory [48]. Chromatin released from mesangial cells into the mesangial matrix or glomerular basement membrane may bind circulating anti-dsDNA ab and thereby form the immune complexes observed as electron dense structures [48]. However, released chromatin without autoantibodies has never been observed in the mesangial matrix or the GMB of lupus prone mice or in biopsies of SLE patients. Immune complexes may be actively cleared by mesangial cells into the mesangial matrix [49]. Due to the affinity of nucleosomes towards the matrix components a consequent accumulation of

immune complexes are a more likely mechanism and may be named the "planted immune complex" theory. The immune complexes may activate the mesangial cells through FcRs or TLRs to produce cytokines and chemokines that will attract infiltrating cells [7](see below). We have recently demonstrated that nucleosomes alone can activate mesangial cells to produce cytokines and chemokines [50]. Other cells nearby like the glomerular endothelial cells and podocytes are believed to contribute to the subendothelial and subepithelial deposits observed within the GBM. This theory has never been proven, and we speculate that it is more likely that the subendothelial and subepithelial depositions is due to the pressure of the blood flow forcing the immune complexes more and more towards the epithelial side of the GMB were they are stopped against the podocytes.

Tubular cells and microcapillary endothelial cells

Other renal cells include the different tubular cells and the endothelial cells in arteries and capillaries within the renal circulation. Apoptosis of tubular cells has been detected in human kidney biopsies, but the levels were found to be too low to be considered as a reasonable quantitative source for autoantibody binding and immune complex formation [46]. The capillary endothelial cells are the most likely candidate for the source of released chromatin fragments of the residential kidney cells. They are in direct contact with the circulating anti-dsDNA antibodies/nucleosomes and the activated effector cells. Nucleosomes have been shown to activate endothelial cell to produce pro-inflammatory cytokines like IL6 and IL8 [51]. In addition endothelial bound nucleosomes and C1q are targets for autoantibodies and lead to activation of complement [52]. Endothelial cells are activated during inflammatory processes and may release microparticles containing chromatin fragments that bind anti-dsDNA antibodies [53;54]. Staining with anti-Meca32 and endoglin(CD105) antibodies on kidney sections of NZBWF1 mice revealed that immune complex deposits in the mesangial matrix contained Meca32 and endoglin (Figure 1) during different stages of the disease. Endoglin is known to be expressed on activated endothelial cells thereby explaining the staining within the glomeruli. Meca 32 is not expressed on glomerular endothelial cells. This may point at dead endothelial cells or released endothelial microparticles as a source of the chromatin found in immune complex depositions [55]. However,

the release of microparticles into the circulation is not restricted to the kidney and may origin from endothelial cells outside the kidneys.

Infiltrating granulocytes, macrophages, T and B cells

Polymorphonuclear leukocytes (granulocytes) are normally the first cells recruited at sites of inflammation [56]. Nucleosomes have been shown to induce activation and secretion of pro-inflammatory cytokines by PMN [57]. Netting neutrophils have recently been demonstrated to induce endothelial damage, infiltrate tissue and expose immunological stimulatory molecules in SLE patients [58]. However, their role in lupus nephritis was not investigated [58]. Impairment in clearance of nets by reduced degradation is associated with proteinuria in lupus patients [41]. An imbalance in proapoptotic and antiapoptotic factors was shown in both neutrophils and sera from patients with juvenile-onset SLE, leading to increased neutrophil apoptosis in these patients [59]. Mice studies have not revealed the role of neutrophils as the mice have small amount of these cells in the circulation in comparison to humans [56]. However we found an increase in infiltrating granulocytes within the glomeruli and interstitial between tubuli in B/W mice with anti-dsDNA antibodies and proteinuric mice compared with young mice, although no indication that these cells are the origin of deposited chromatin [50].

Macrophages are essential in the clearance of apoptotic cell debris and immune complexes. Studies have shown that induction of apoptosis in circulating macrophages by chlodronate filled liposomes accelerated the development of lupus nephritis in lupus prone mice [60]. This was possible due to a subsequent decrease in the clearance of apoptotic cells and thereby increasing the amount of circulating chromatin [60]. There are no known studies on the role of increased cell death of T cells in lupus nephritis. There are however an enormous amount of infiltrating T cells and macrophages within the kidney of lupus prone mice and SLE patients. The T cells infiltrating glomeruli in lupus prone mice are CD3+ T cells (Figure 1) of all classes, Th1, Th2 and Th17 cells [61]. The fate if these cells during the disease have not been investigated, but considering the amount and the life span of these helper and effector cells, they may be a source of released chromatin. Apoptosis of B cells have been demonstrated to accelerate lupus nephritis in lupus prone mice [62]. Studies of B cell infiltration has revealed that this happens only after immune complex deposition and is mainly located in tertiary lymphoid

structures within the kidney and not in glomeruli and interstitial between tubules. However, B cells are essential for driving the disease from silent mesangial nephritis to end stage disease [63].

Concluding hypothesis

Production of anti-dsDNA ab, formation of immune complex, and their subsequent deposition within the mesangial matrix activate mesangial cells to produce cytokines and chemokines thereby attracting immune cells. Circulating immune complexes activate endothelial cells and may release chromatin containing microparticles that, if not properly cleared, may act as antigen for autoantibodies. This will contribute to the amount of immune complexes formed. The infiltration of effector cells accelerates the inflammation and tissue damage within the glomeruli and activate endothelial cells. This will recruit and maintain lymphocyte infiltration into the interstitium. Increase in inflammation will increase the amount of dying cells within the kidney whatever origin they have, and lead to excess amount of released chromatin that amplify the disease process. The exact mechanism(s) leading to breakage of tolerance and activation of autoantigen specific lymphocytes are still unknown. Novel strategies for the prevention of lupus nephritis may depend on the further investigations of programmed cell death and the role of circulating chromatin in SLE.

Acknowledgement

This work was supported by Foundation for Health and Rehabilitation through the Norwegian Rheumatology Organization (project 2008/2/0229).

Reference List

1. Kaliyaperumal A, Mohan C, Wu W, Datta SK. Nucleosomal peptide epitopes for nephritis-inducing T helper cells of murine lupus. Journal of Experimental Medicine 1996; 183:2459-69.

2. DesaiMehta A, Mao CC, Rajagopalan S, Robinson T, Datta SK. Structure and Specificity of T-Cell Receptors Expressed by Potentially Pathogenic Anti-Dna Autoantibody-Inducing T-Cells in Human Lupus. Journal of Clinical Investigation 1995; 95:531-41.

3. Kalaaji M, Fenton KA, Mortensen ES *et al*. Glomerular apoptotic nucleosomes are central target structures for nephritogenic antibodies in human SLE nephritis. Kidney Int. 2007; 71:664-72.

4. Kalaaji M, Mortensen E, Jorgensen L, Olsen R, Rekvig OP. Nephritogenic lupus antibodies recognize glomerular basement membrane-associated chromatin fragments released from apoptotic intraglomerular cells. Am.J.Pathol. 2006; 168:1779-92.

5. Hedberg A, Fismen S, Fenton KA, Mortensen ES, Rekvig OP. Deposition of chromatin-IgG complexes in skin of nephritic MRL-lpr/lpr mice is associated with increased local matrix metalloprotease activities. Exp.Dermatol. 2010; 19:e265-e274.

6. Fismen S, Hedberg A, Fenton KA *et al*. Circulating chromatin-anti-chromatin antibody complexes bind with high affinity to dermo-epidermal structures in murine and human lupus nephritis. Lupus 2009; 18:597-607.

7. Lech M, Anders HJ. The pathogenesis of lupus nephritis. J.Am.Soc.Nephrol. 2013; 24:1357-66.

8. Mjelle JE, Rekvig OP, Fenton KA. Nucleosomes possess a high affinity for glomerular laminin and collagen IV and bind nephritogenic antibodies in murine lupus-like nephritis. Ann.Rheum.Dis. 2007; 66:1661-8.

9. Mjelle JE, Rekvig OP, van der Vlag J, Fenton KA. Nephritogenic antibodies bind in glomeruli through interaction with exposed chromatin fragments and not with renal cross-reactive antigens. Autoimmunity 2011; 44:373-83.

10. Fenton K, Fismen S, Hedberg A *et al*. Anti-dsDNA antibodies promote initiation, and acquired loss of renal Dnase1 promotes progression of lupus nephritis in autoimmune (NZBxNZW)F1 mice. PLoS.One. 2009; 4:e8474.

11. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 1997; 389:251-60.

12. Tan S, Davey CA. Nucleosome structural studies. Curr.Opin.Struct.Biol. 2011; 21:128-36.

13. Jorgensen MH, Rekvig OP, Jacobsen RS, Jacobsen S, Fenton KA. Circulating levels of chromatin fragments are inversely correlated with anti-dsDNA antibody levels in human and murine systemic lupus erythematosus. Immunol.Lett. 2011; 138:179-86.

14. Hedberg A, Fismen S, Fenton KA *et al*. Heparin exerts a dual effect on murine lupus nephritis by enhancing enzymatic chromatin degradation and preventing chromatin binding in glomerular membranes. Arthritis Rheum. 2011; 63:1065-75.

15. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. Nat.Rev.Mol.Cell Biol. 2008; 9:231-41.

16. Opferman JT, Korsmeyer SJ. Apoptosis in the development and maintenance of the immune system. Nat.Immunol. 2003; 4:410-5.

17. Fuchs Y, Steller H. Programmed cell death in animal development and disease. Cell 2011; 147:742-58.

18. Siegel RM. Caspases at the crossroads of immune-cell life and death. Nat.Rev.Immunol. 2006; 6:308-17.

19. Radic M, Marion T, Monestier M. Nucleosomes are exposed at the cell surface in apoptosis. J.Immunol. 2004; 172:6692-700.

20. Munoz LE, Lauber K, Schiller M, Manfredi AA, Herrmann M. The role of defective clearance of apoptotic cells in systemic autoimmunity. Nat.Rev.Rheumatol. 2010; 6:280-9.

21. Kaczmarek A, Vandenabeele P, Krysko DV. Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. Immunity. 2013; 38:209-23.

22. Fullgrabe J, Hajji N, Joseph B. Cracking the death code: apoptosis-related histone modifications. Cell Death.Differ. 2010; 17:1238-43.

23. van Bavel CC, Dieker JW, Kroeze Y *et al*. Apoptosis-induced histone H3 methylation is targeted by autoantibodies in systemic lupus erythematosus. Ann.Rheum.Dis. 2011; 70:201-7.

24. van Bavel CC, Dieker JW, Tamboer WP, van der Vlag J, Berden JH. Lupus-derived monoclonal autoantibodies against apoptotic chromatin recognize acetylated conformational epitopes. Mol.Immunol. 2010; 48:248-56.

25. Migliorini A, Anders HJ. A novel pathogenetic concept-antiviral immunity in lupus nephritis. Nat.Rev.Nephrol. 2012; 8:183-9.

26. Fransen JH, Hilbrands LB, Ruben J *et al*. Mouse dendritic cells matured by ingestion of apoptotic blebs induce T cells to produce interleukin-17. Arthritis Rheum. 2009; 60:2304-13.

27. Golstein P, Kroemer G. Cell death by necrosis: towards a molecular definition. Trends Biochem.Sci. 2007; 32:37-43.

28. Linkermann A, Green DR. Necroptosis. N.Engl.J.Med 2014; 370:455-65.

29. Ofengeim D, Yuan J. Regulation of RIP1 kinase signalling at the crossroads of inflammation and cell death. Nat.Rev.Mol.Cell Biol. 2013; 14:727-36.

30. Ch'en IL, Tsau JS, Molkentin JD, Komatsu M, Hedrick SM. Mechanisms of necroptosis in T cells. J.Exp.Med 2011; 208:633-41.

31. Laster SM, Wood JG, Gooding LR. Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. J.Immunol. 1988; 141:2629-34.

32. Han J, Zhong CQ, Zhang DW. Programmed necrosis: backup to and competitor with apoptosis in the immune system. Nat.Immunol. 2011; 12:1143-9.

33. Sanz AB, Izquierdo MC, Sanchez-Nino MD *et al*. TWEAK and the progression of renal disease: clinical translation. Nephrol.Dial.Transplant. 2014; 29 Suppl 1:i54-i62.

34. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. Cell 2011; 147:728-41.

35. Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. Nat.Rev.Immunol. 2013; 13:722-37.

36. Zhou XJ, Cheng FJ, Zhang H. Emerging View of Autophagy in Systemic Lupus Erythematosus. Int.Rev.Immunol. 2014.

37. Clarke AJ, Ellinghaus U, Cortini A *et al*. Autophagy is activated in systemic lupus erythematosus and required for plasmablast development. Ann.Rheum.Dis. 2014.

38. Gros F, Arnold J, Page N *et al*. Macroautophagy is deregulated in murine and human lupus T lymphocytes. Autophagy. 2012; 8:1113-23.

39. Brinkmann V, Reichard U, Goosmann C *et al*. Neutrophil extracellular traps kill bacteria. Science 2004; 303:1532-5.

40. Brinkmann V, Zychlinsky A. Beneficial suicide: why neutrophils die to make NETs. Nat.Rev.Microbiol. 2007; 5:577-82.

41. 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; 107:9813-8.

42. Lande R, Ganguly D, Facchinetti V *et al*. Neutrophils Activate Plasmacytoid Dendritic Cells by Releasing Self-DNA-Peptide Complexes in Systemic Lupus Erythematosus. Sci.Transl.Med 2011; 3:73ra19.

43. Garcia-Romo GS, Caielli S, Vega B *et al*. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci.Transl.Med 2011; 3:73ra20.

44. Cui JH, Qiao Q, Guo Y *et al*. Increased apoptosis and expression of FasL, Bax and caspase-3 in human lupus nephritis class II and IV. J.Nephrol. 2012; 25:255-61.

45. Zheng L, Sinniah R, Hsu SI. Renal cell apoptosis and proliferation may be linked to nuclear factor-kappaB activation and expression of inducible nitric oxide synthase in patients with lupus nephritis. Hum.Pathol. 2006; 37:637-47.

46. Faurschou M, Penkowa M, Andersen CB, Starklint H, Jacobsen S. Renal cell apoptosis in human lupus nephritis: a histological study. Lupus 2009; 18:994-9.

47. Seredkina N, Zykova SN, Rekvig OP. Progression of murine lupus nephritis is linked to acquired renal Dnase1 deficiency and not to up-regulated apoptosis. Am.J.Pathol. 2009; 175:97-106.

48. de Zubiria SA, Herrera-Diaz C. Lupus nephritis: an overview of recent findings. Autoimmune.Dis. 2012; 2012:849684.

49. Schlondorff D, Banas B. The mesangial cell revisited: no cell is an island. J.Am.Soc.Nephrol. 2009; 20:1179-87.

50. Kanapathippillai P, Hedberg A, Fenton CG, Fenton KA. Nucleosomes contribute to increase mesangial cell chemokine expression during the development of lupus nephritis. Cytokine 2013; 62:244-52.

51. Tanner JE. Nucleosomes activate NF-kappaB in endothelial cells for induction of the proangiogenic cytokine IL-8. Int.J.Cancer 2004; 112:155-60.

52. O'Flynn J, Flierman R, van der Pol P *et al*. Nucleosomes and C1q bound to glomerular endothelial cells serve as targets for autoantibodies and determine complement activation. Mol.Immunol. 2011; 49:75-83.

53. Ostergaard O, Nielsen CT, Iversen LV *et al*. Unique protein signature of circulating microparticles in systemic lupus erythematosus. Arthritis Rheum. 2013; 65:2680-90.

54. Nielsen CT, Ostergaard O, Stener L *et al*. Increased IgG on cell-derived plasma microparticles in systemic lupus erythematosus is associated with autoantibodies and complement activation. Arthritis Rheum. 2012; 64:1227-36.

55. Pisetsky DS, Gauley J, Ullal AJ. Microparticles as a source of extracellular DNA. Immunol.Res. 2011; 49:227-34.

56. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat.Rev.Immunol. 2013; 13:159-75.

57. Lindau D, Ronnefarth V, Erbacher A, Rammensee HG, Decker P. Nucleosome-induced neutrophil activation occurs independently of TLR9 and endosomal acidification: implications for systemic lupus erythematosus. Eur.J.Immunol. 2011; 41:669-81.

58. Villanueva E, Yalavarthi S, Berthier CC *et al*. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J.Immunol. 2011; 187:538-52.

59. Midgley A, McLaren Z, Moots RJ, Edwards SW, Beresford MW. The role of neutrophil apoptosis in juvenile-onset systemic lupus erythematosus. Arthritis Rheum. 2009; 60:2390-401.

60. Denny MF, Chandaroy P, Killen PD *et al*. Accelerated macrophage apoptosis induces autoantibody formation and organ damage in systemic lupus erythematosus. J.Immunol. 2006; 176:2095-104.

61. Apostolidis SA, Crispin JC, Tsokos GC. IL-17-producing T cells in lupus nephritis. Lupus 2011; 20:120-4.

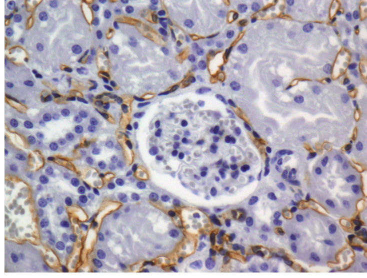
62. Trebeden-Negre H, Weill B, Fournier C, Batteux F. B cell apoptosis accelerates the onset of murine lupus. European Journal of Immunology 2003; 33:1603-12.

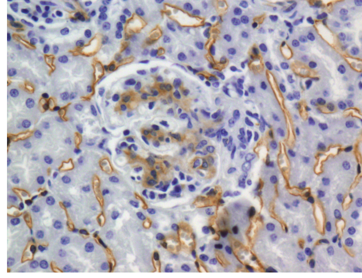
63. Teichmann LL, Schenten D, Medzhitov R, Kashgarian M, Shlomchik MJ. Signals via the Adaptor MyD88 in B Cells and DCs Make Distinct and Synergistic Contributions to Immune Activation and Tissue Damage in Lupus. Immunity. 2013; 38:528-40.

Figure 1. Glomerular detection of autologous cells as source of released chromatin. Kidney sections of lupus prone NZB/WF1 mice at different ages (4 and 34 w.o) were stained with anti-Meca32, anti-endoglin (CD105), anti-F480 (all from BioLegend, San Diego, CA, USA), anti-CD3 (Dako, Glostrup, Denmark) antibodies detected by IHC using Polink-2 Plus HRP detection kits for tissue (Golden Bridge International, Inc, Mukilteo, WA, USA) according to protocol described in details in [50]. All pictures are taken at 400 x , scale bar= 50µm.

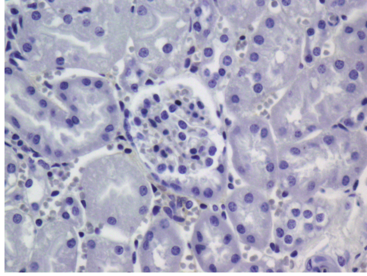
4 w.o NZB/W | 34 w.o NZB/W

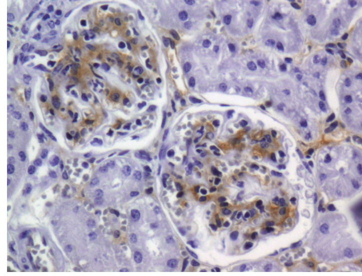
Meca 32



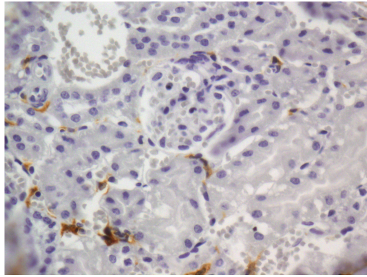


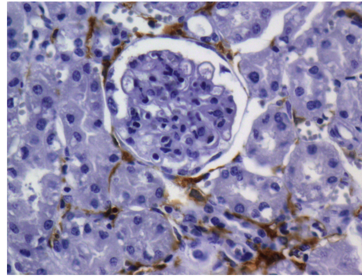
Endoglin (CD105)





F480





CD3