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Levels of soluble VCAM-1, soluble ICAM-1, and soluble E-selectin during disease exacerbations in patients with systemic lupus erythematosus (SLE); a long term prospective study

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SUMMARY

Active SLE is characterized by immune deposits and subsequent vascular inflammation in many organs. Expression and up-regulation of adhesion molecules is basic to migration of inflammatory cells into the tissues. Recently, soluble isoforms of these molecules have been described which might be an expression of their up-regulation in the tissues and, as such, of disease activity. The purpose of this study was to evaluate whether changes in levels of soluble adhesion molecules reflect disease activity. We analysed serial sera in a 6-month period preceding 22 consecutive exacerbations of SLE for levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), and sE-selectin. Levels were related to clinical disease activity (SLEDAI), and levels of anti-dsDNA and complement. At the time of maximal disease activity, levels of sVCAM-1 in patients with SLE were higher than those in controls ($P < 0{\cdot}0001$), levels in patients with renal involvement being higher than in those without ($P < 0{\cdot}02$). Levels of sVCAM-1 correlated with SLEDAI scores ($P < 0{\cdot}05$) and, inversely, with levels of C3 ($P = 0{\cdot}01$). In addition, in the presence of anti-dsDNA, levels of sVCAM-1 tended to correlate with levels of these autoantibodies ($P < 0{\cdot}1$). Levels of sICAM-1 were normal and sE-selectin levels even decreased compared with controls. Levels of sVCAM-1 were higher at the moment of relapse ($P = 0{\cdot}001$) than at 6 months before this time point. This rise correlated with the rise in SLEDAI score ($P < 0{\cdot}02$). Levels of sICAM-1 and sE-selectin did not rise, and remained in the normal range in all exacerbations studied. In conclusion, in contrast to sICAM-1 and sE-selectin, levels of sVCAM-1 are increased, rise parallel to disease activity during exacerbations in SLE, and are associated with decreasing levels of complement factors. This favours the hypothesis of immune deposit formation, activation of the complement cascade and activation of endothelial cells. Concurrent up-regulation of vascular adhesion molecules may thus result in transmigration of activated inflammatory cells inducing tissue damage.

Keywords systemic lupus erythematosus soluble adhesion molecules prospective study

INTRODUCTION

Adhesion molecules are essential for cellular interactions between immunocompetent cells, and play an important role in cellular activation and adhesion[1,2]. E-selectin, previously also known as ELAM-1, is expressed exclusively on endothelial cells after stimulation with cytokines *in vitro* [3]. Like all selectins, it interacts with carbohydrate structures on circulating cells, thereby initiating the so-called 'rolling' of leucocytes on the endothelial surface [1], with subsequent up-regulation of the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on the endothelial surface. Concurrently, β_1 (part of very late antigen-4 (VLA-4)) and β_2 (part of leucocyte function antigen-1 (LFA-1)) integrin molecules are induced on mononuclear cells and neutrophils, respectively [2]. The binding of these integrins to their cellular ligands, i.e. VLA-4/VCAM-1 and LFA-1/ICAM-1 interactions, results in the firm attachment and transmigration of the cells involved. Studies in animals and *in vitro* experiments have suggested a differential expression of E-selectin, ICAM-1, and VCAM-1 upon stimulation with different combinations of cytokines [4,5].

SLE is an autoimmune disease with putative small vessel

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involvement in many organ systems. Persistently activated lymphocytes and a wide range of autoantibodies are detectable in the circulation [6,7]. Particularly, autoantibodies directed against double-stranded DNA (anti-dsDNA) are very specific for the disease, fluctuate with disease activity [8], and are thought to be important in the pathogenesis of the disease by their participation in the forming of immune deposits. Subsequent activation of the complement system results in endothelial activation and concurrent chemoattraction of immunocompetent cells. These events induce the up-regulation of adhesion molecules, thus promoting the transmigration of inflammatory cells and, eventually, organ damage.

Indeed, increased expression of ICAM-1 and VCAM-1 was found in the glomerular mesangium and on the endothelium in autoimmune murine lupus nephritis [9,10]. Pallis *et al.* reported increased expression of E-selectin, ICAM-1, and, in the case of lymphocytic infiltrates, VCAM-1 in skeletal muscle biopsies from patients with SLE [11]. In addition, high expression of VCAM-1, ICAM-1, and E-selectin was found on endothelial cells in biopsy specimens from non-lesional skin in patients with active SLE [12].

Recently, soluble isoforms of adhesion molecules have been reported that are capable of binding to their respective ligands after stimulation of endothelial cells with cytokines *in vitro* [13–15]. Levels of these isoforms may reflect enhanced expression of adhesion molecules on immunocompetent and endothelial cells. A number of studies have dealt with this subject in SLE [14,16–19]. These were all cross-sectional studies on unselected SLE patients, while clinical characteristics of the patients involved in the studies were not clearly defined.

To investigate the possible use of those molecules as markers of disease activity, we measured levels of soluble VCAM-1 (sVCAM-1), soluble ICAM-1 (sICAM-1), and soluble E-selectin (sE-selectin) before and during 22 disease exacerbations in SLE. Levels of these molecules were related to clinical disease activity and organ involvement, levels of anti-dsDNA, and complement factors C1q, C3, and C4.

PATIENTS AND METHODS

Patients

This study concerns a cohort of 84 outclinic patients (74 females, 10 males), fulfilling the 1982 revised ARA criteria for the diagnosis of SLE [20], who participate in a prospective long-term clinical follow-up study [8]. All 84 patients are seen at least at 3-month intervals at the outclinic. The SLE disease activity index (SLEDAI) [21] is calculated at every outclinic visit from signs and symptoms recorded according to a protocol and routine laboratory tests. Dosages of prednisolone and/or immunosuppressives are recorded. Blood samples are drawn in EDTA monthly and plasma is stored at −80°C. Exacerbations are defined as described previously [8].

Special attention is paid to the occurrence of infections in order to discriminate between symptoms and/or signs attributable to infection and those attributable to lupus activity. An infection is considered: (i) *proven*: in case a positive culture and/or serological evidence is obtained in combination with clinical symptoms suggestive of an infection. Herpes zoster is considered on the presence of a typical clinical presentation only. A urinary tract infection is considered when symptomatic and accompanied by leucocyturia and when > 100 000 colonies/ml are grown; (ii) *probable*: in case positive cultures or positive serological tests are not obtained, but clinical symptoms strongly suggest an infection and are followed by a prompt recovery after the institution of antimicrobial therapy.

Out of the 84 patients involved in our follow-up study, the first 22 consecutive patients (19 females and three males) who developed an exacerbation without concomitant infection were analysed in this study. In this group of patients the mean age at the start of the study was 32 years (range 20–50 years), and SLE was diagnosed a mean 7 years (range 0–27 years) before the start of the study. Plasma samples taken at 6, 3, 2, and 1 month before, and at the moment of maximal disease activity were analysed for levels of sVCAM-1, sICAM-1, sE-selectin, anti-dsDNA, C1q, C3, and C4.

Methods

Plasma levels of sVCAM-1, sICAM-1, and sE-selectin were analysed by sandwich ELISA according to the instructions of the manufacturer (British Biotechnology Products Ltd, Abingdon, UK). All measurements were done with the same batch, and in duplicate. In brief, microtitre plates were coated with a murine IgG class MoAb directed against one epitope on sVCAM-1, sICAM-1, or sE-selectin, respectively. After incubation with plasma samples or standards in appropriate dilution, a biotinylated murine IgG class MoAb directed against a second epitope on sVCAM-1, sICAM-1, and sE-selectin, respectively, was added. After addition of streptavidin-conjugated horseradish peroxidase (HRP), colour reaction was obtained by tetramethylbenzidine, and the plates were read on an automated multiscanner. Interassay and intra-assay coefficients of variation were 8% and 6%, respectively. To determine the normal range, control samples were obtained from 57 healthy individuals. Their mean values (± s.d.) for sVCAM-1, sICAM-1, and sE-selectin were 588 ± 159 ng/ml, 328 ± 77·4 ng/ml, and 42·7 ± 13·6 ng/ml, respectively. Levels were not related to the age or sex of controls. The mean ± 2 s.d. from this control group was taken as the normal range.

Anti-dsDNA antibodies were detected by Farr assay using ^{125}I-labelled recombinant ds-DNA (Diagnostic Products Corporation, Los Angeles, CA) which is free from contamination with ssDNA. Farr assay was performed according to the manufacturer's instructions, and positive samples were measured at different dilutions to obtain measurements within the range of the assay. C1q does not interfere with this assay. Results of this assay were expressed in U/ml, using Wo/80 as the ultimate standard [22]. The normal value of this Farr assay in our laboratory is $\leqslant$ 15 U/ml; intra- and interassay variation are both less than 10%. Levels of complement factors C1q, C3, and C4 were measured by Mancini immunodiffusion technique.

Statistical analysis

Analysis was done using the SYSTAT statistical package. Differences in parameters between groups were evaluated with the *t*-test. For analysing changes in study parameters, paired *t*-test was performed. Spearman's test was applied for detecting correlations between different study parameters. If normal distribution could not be assumed, Mann–Whitney *U*-test or Wilcoxon's paired rank-sum test were applied. $P < 0{\cdot}05$ (two-sided) was considered significant.

Table 1. Characteristics of 22 disease exacerbations in patients with SLE

	Number of patients with symptom present	
	Major exacerbations ($n = 15$)	Minor exacerbations ($n = 7$)
Renal involvement	10	1
Involvement of CNS	7	0
Skin involvement	6	2
Musculoskeletal involvement	4	6
Serositis	1	6
Haematological abnormalities	8	3
Vasculitis	1	1
Fever	2	3

CNS, Central nervous system.

RESULTS

For this study the first 22 consecutive exacerbations (15 major and seven minor) in different individuals were evaluated. Only exacerbations without evidence of an infection at the time of maximal disease activity were included. Characteristics of these exacerbations are shown in Table 1. Six patients used immunosuppressive medication at constant and, generally, low dosage in the period preceding the exacerbation, i.e. prednisolone ($n = 3$, range 7·5–10 mg/day), or azathioprine ($n = 5$, range 25–150 mg/day). No differences were found regarding levels of sVCAM-1, sICAM-1, or sE-selectin in the patients using prednisolone and/or azathioprine in comparison with those without.

Levels of soluble adhesion molecules at the time of maximal disease activity

At the time of maximal disease activity during exacerbations, plasma levels of sVCAM-1 (median level 926 ng/ml, range 446–2592 ng/ml; $P < 0·0001$) were higher in patients than in controls, whereas levels of sICAM-1 (median 269 ng/ml, range 155–597 ng/ml) were not different from those of controls, and levels of sE-selectin (median 30·2 ng/ml, range 19·8–60·9 ng/ml) were even lower ($P = 0·01$) (Fig. 1). Both major and minor exacerbations showed significantly increased levels of sVCAM-1 (both $P < 0·001$). Levels of sICAM-1 and sE-selectin were not different from controls in those subgroups. sVCAM-1 levels were higher during exacerbations with renal involvement (median 1557 ng/ml, range 561–2305 ng/ml) than in those without (median 770 ng/ml, range 446–2592 ng/ml; $P < 0·02$). At the moment of maximal disease activity, levels of sVCAM-1 correlated with SLEDAI disease activity scores ($r = 0·50$, $P < 0·05$) (Fig. 2), were inversely related to complement factor C3 ($r = -0·56$, $P = 0·01$), and, in the presence of anti-dsDNA, tended to correlate with levels of these antibodies ($P < 0·1$) (Fig. 3). No relation with levels of complement factors C1q or C4 could be found. No intercorrelations were found between levels of the three soluble adhesion molecules studied, nor between levels of sICAM-1or sE-selectin, and levels of anti-dsDNA, complement factors, or disease activity scores.

Serial measurements of soluble adhesion molecules in the time period preceding the moment of maximal disease activity

Longitudinal samples were available in 19 (12 major and seven minor) out of the 22 exacerbations studied. Levels of sVCAM-1 in patients were higher than those of controls at all time points studied ($P < 0·005$), except at 6 months before the moment of maximal disease activity (Fig. 4). As for exacerbations with renal involvement ($n = 8$), levels of sVCAM-1 were increased at this time point as well ($P < 0·005$). Analysis of individual changes in levels of sVCAM-1 revealed a significant rise of these levels during the study period ($P = 0·001$). This rise occurred predominantly in the period between 6 and 3 months ($P = 0·01$), and during the 1-month period before the exacerbation ($P < 0·05$). Changes in sVCAM-1 levels during the study period correlated with changes in SLEDAI disease activity scores ($r = 0·54$, $P < 0·02$), but not with changes in levels of anti-dsDNA or complement factors. During three exacerbations, anti-dsDNA levels remained in the normal range. Levels of sVCAM-1, however, rose during those exacerbations, and complement levels dropped.

Levels of sICAM-1 and sE-selectin remained in the normal range during the period preceding the relapse (Fig. 4). Changes in levels of those molecules were not related to particular organ involvement.

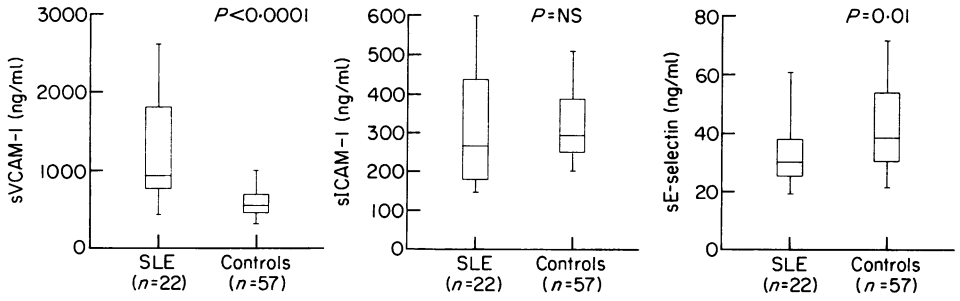

Fig. 1. Box plots indicating range (whiskers), 25–75% interval (box), and median value (horizontal line) of serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), and sE-selectin in 22 consecutive patients with SLE at the moment of maximal disease activity during an exacerbation, and in 57 healthy controls. Levels of sVCAM-1 are significantly higher ($P < 0·0001$), whereas levels of sICAM-1 are comparable to ($P =$ NS), and sE-selectin levels are even lower ($P = 0·01$) than controls.

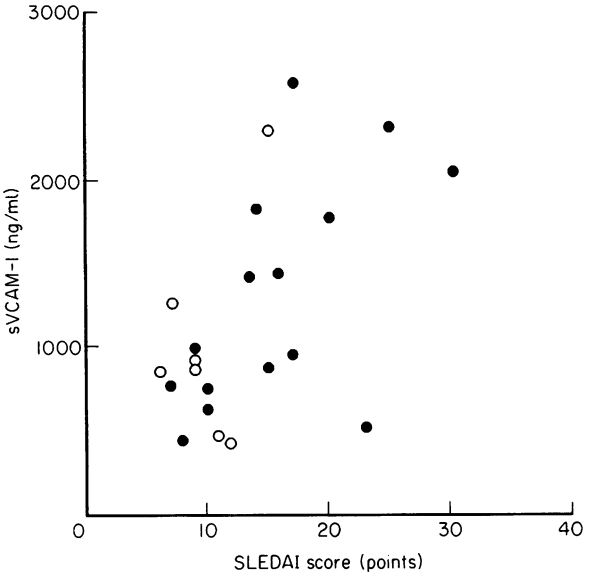

Fig. 2. Correlation between levels of soluble intercellular adhesion molecule-1 (sVCAM-1) and disease activity scores (SLEDAI) at the moment of maximal disease activity during 22 exacerbations in 22 consecutive patients with SLE. ○, Minor exacerbation ($n = 7$); ●, major exacerbation ($n = 15$).

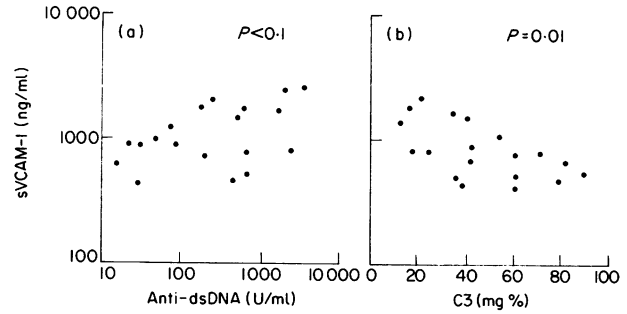

Fig. 3. Correlation between levels of soluble intercellular adhesion molecule-1 (sVCAM-1) and levels of anti-dsDNA (a), and levels of complement C3 (b) at the moment of maximal disease activity during 19 anti-dsDNA-positive exacerbations in 22 consecutive patients with SLE.

DISCUSSION

In this study, we analysed levels of soluble adhesion molecules during 22 disease exacerbations in patients with SLE. The aim was to analyse the possible relation between clinical lupus activity and levels of soluble adhesion molecules. Hence, we tried to exlude other factors that might influence those levels, in particular infections. We found increased levels of sVCAM-1, which is in agreement with one other study on this subject [15]. Increased levels were particularly observed during exacerbations with renal involvement. Levels rose significantly up to the moment of maximal disease activity, whereas levels of sICAM-1 and sE-selectin remained in the normal range. Levels of sVCAM-1 were related to clinical disease activity scores, anti-dsDNA levels, and, inversely, to levels of complement C3. These findings are consistent with the hypothesis that immune complex formation and up-regulation of adhesion molecules are intimately connected in SLE, and are involved in the pathogenesis of the disease. As activation of the complement cascade follows the classical pathway, one would expect a relation between levels of sVCAM-1 and those of both C3 and C4. In the present study, however, no relation between levels of sVCAM-1 and C4 could be found. Although this discrepancy between levels of C3 and C4 can not be clearly explained, other clinical studies in SLE also have yielded conflicting results with respect to the relation between disease activity and levels of C3 and C4, either stating that C4 is superior to C3 in monitoring disease activity in SLE [23], or the opposite [24]. Local formation and/or deposition of immune complexes with subsequent activation of the endothelium may result in increased expression of adhesion molecules. Assuming that plasma levels of sVCAM-1 reflect expression of VCAM-1 as a result of endothelial activation, a rise in levels of this soluble isoform suggests increased expression of VCAM-1 in the tissues. This will result in the adherence and subsequent migration of mononuclear cells, in particular when these cells are activated. Indeed, expression of activation markers is up-regulated on circulating B and T cells already during quiescent SLE [6]. Before disease activity, this expression is further increased [25]. Enhanced expression of VCAM-1 on the endothelium may facilitate the transmigration of monocytes and $CD4^+$ memory/effector cells, since these cells express VLA-4, the ligand for VCAM-1, whereas 'naive' $CD4^+$ lymhocytes do not [26]. These effector mononuclear cells may enhance or sustain the expression of VCAM-1 as a result of local cytokine production [27], and influence the expression of other adhesion molecules as well. This change in the extracellular milieu may result in the local accumulation of immunocompetent cells and subsequent tissue damage. Indeed, in contrast to normal kidneys, monocytes and lymphocytes are present in renal biopsy specimens of patients with active lupus nephritis [28].

In this study we found higher levels of sVCAM-1 in exacerbations with renal disease compared with those without. This can hardly be explained by impaired renal clearance because of the high molecular weight of sVCAM-1. In agreement, calculated corrections for reduced creatinin clearance did not alter the results. Possible explanations for the occurrence of higher sVCAM-1 levels in patients with renal involvement than in those without include the large endothelial surface of the kidneys, as well as additional release of sVCAM-1 by other than endothelial cells. In biopsy specimens from patients with lupus nephritis, VCAM-1 expression has been reported to occur on various renal components, i.e. parietal epithelium, glomerular capillary wall, mesangium, and tubular epithelial cells [29]. Although VCAM-1 is constitutively expressed in the normal kidney [30], this expression is increased in immune-mediated renal disease [31]. In addition, high VCAM-1 expression was found on endothelium, arterial smooth muscle, and mesangium cells in transplant rejection biopsy specimens [31]. In our study, major disease activity was present in almost all exacerbations with renal involvement, suggesting that extensive up-regulation of VCAM-1 was present in these patients. This is of interest in view of our finding that high levels of sVCAM-1 are related to high levels of anti-dsDNA and low complement levels, which confirms previous data. Taken together, the expression of VCAM-1 may be increased by immune deposit formation, and allows VLA-4-bearing monocytes and effector lymphocytes to migrate into the renal tissue and contribute to the pathogenesis of lupus nephritis.

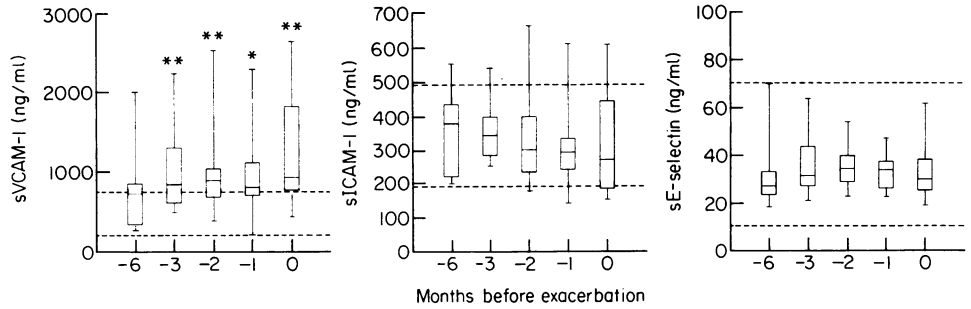

Fig. 4. Plasma levels of soluble intercellular adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1) and sE-selectin in 19 patients with SLE during follow up at 6, 3, 2, and 1 month before and at the moment of disease exacerbation. Horizontal dashed lines denote lower and upper limit of normal values. See Fig. 1 for explanation of box. $*P = 0{\cdot}002$; $**P < 0{\cdot}001$ compared with healthy controls.

In this study, levels of sICAM-1 and sE-selectin were predominantly within the normal range at the moment of maximal disease activity, and did not change in the time period before relapse. These findings might in part be explained by binding of the functionally active soluble molecules to their respective ligands on activated leucocytes. The present finding of high sVCAM-1 levels, however, contrasts with this hypothesis, although it might be due to an excess of released molecules compared with the number of VLA-4 molecules accessible. In case of E-selectin, the short time period of expression of this molecule on the endothelial surface after activation may account for normal levels of circulating E-selectin as well. Increased levels of sICAM-1 in unselected SLE patients have been described [16], although other studies have reported levels of this molecule to be comparable to controls in unselected SLE patients [17,18]. Differences in disease activity, the presence of infections, and differences in the assay used may explain these discrepancies, as all of the former studies were non-protocollized cross-sectional studies. In one study [19] levels of sE-selectin have been reported increased, both in active and inactive SLE, compared with matched controls [19]. This result contrasts with our present longitudinal observations. Although differences in methodology might be responsible for these contrasting findings, Bruijn *et al.* [29] reported only faint glomerular expression of E-selectin in biopsy specimens of patients with lupus nephritis. In addition, in patients with primary vasculitides, levels of sE-selectin were found to be in the normal range during disease exacerbations [32].

The specific pathophysiological role of circulating forms of adhesion molecules remains to be elucidated. Since soluble adhesion molecules are functionally active *in vitro* [13–15], binding of these isoforms to their ligands on activated immunocompetent cells might dampen an active immune response. MoAbs antibodies directed against VCAM-1 or VLA-4 inhibit the adhesiveness of T cell and macrophage cell lines to cytokine-activated MRL/lpr kidney tissue [9]. In addition, ICAM-1 or LFA-1 targeted antibodies can inhibit experimental autoimmune uveitis [33], and daily treatment of murine cardiac allograft recipients with antibodies directed against VCAM-1 prolongs allograft survival [34]. Interference with adhesion seems of clinical significance. Future studies should reveal whether treatment with molecules that bind to adhesion structures can influence disease activity in patients with SLE.

In conclusion, in contrast to sICAM-1 and sE-selectin, levels of sVCAM-1 are increased, rise parallel to disease activity during exacerbations in SLE, and are associated with decreasing levels of complement factors. This strongly suggests activation of endothelial cells, and favours the hypothesis of immune deposit formation, activation of the complement cascade, and, finally, transmigration of activated inflammatory cells inducing tissue damage.

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