Systemic lupus erythematosus

Autoantibodies to opsonins of apoptotic cells in systemic lupus erythematosus

P C Limburg, M Bijl

Does anti-serum amyloid P component have any clinical relevance?

The major immunological features of systemic lupus erythematosus (SLE) comprise the almost uniform presence of autoantibodies to nuclear antigens and the presence of immune complexes in the blood vessels and tissues, leading to inflammatory reactions. Especially since the studies by Casciola-Rosen and others, the central significance of the process of apoptosis in the aetiology and pathophysiology of SLE is becoming increasingly clear.[1]

Apoptosis or programmed cell death is the major physiological mechanism by which multicellular organisms can reorganise their cellular composition without letting cells die by necrosis, which would lead to the induction of inflammation. Clearance of apoptotic cells depends on structural changes of the apoptotic cell itself, opsonisation of apoptotic cells by several serum proteins, and binding and subsequent phagocytosis of opsonised apoptotic cells by neighbouring phagocytic cells. These processes have been studied in detail, although many questions still remain about the development and expression of disease in SLE.

MOLECULAR CHANGES DURING APOPTOSIS

Apoptosis is usually induced by exogenous stress—for instance, ultraviolet sunlight or deprival of essential growth factors, and leads to the activation of a cascade of proteases (caspases) and other enzymes. This ultimately results in proteolytic cleavage as well as modification of many cellular constituents. Most prominent of these is the chromatin, which contains densely packed DNA in combination with histones and other nuclear proteins, which is cleaved into nucleosomes, the major target of autoantibodies in SLE.

At the level of the cell membrane, structural changes result in the extracellular exposure of normally intracellular phospholipids, as well as release (blebbing) of membrane vesicles. Importantly, these vesicles may contain modified or unmodified intracellular and intranuclear proteins that are the target of the autoimmune response in SLE.[1] This process leads to the binding of opsonising serum proteins and the subsequent binding to phagocytic cells.

OPSONISATION OF APOPTOTIC CELLS

Many soluble factors are known to interact with apoptotic cells, among which complement factors, especially C1q, and phospholipid binding proteins, including the pentraxins and β_2-glycoprotein I, are the most important.[2] These phospholipid binding proteins may either interact directly with phagocytic cells, or may lead to C1q mediated deposition of complement and in this way may interact indirectly.

> ''The opsonins, including the pentraxins, are members of the innate immune systems''

The pentraxin family of proteins comprise the classical short pentraxins—C reactive protein (CRP) and serum amyloid P component (SAP), and the more recently discovered long pentraxins—pentraxin 3 (PTX3) and the neuronal pentraxins (NP1 and NP2) as well as the membrane neuronal pentraxin receptor.[3] Most notably, SAP is a constitutive serum protein, while CRP and PTX3 are produced only during inflammatory conditions (acute phase proteins).

Although the pentraxins may have a common function, they differ in substrate specificity. SAP interacts with phosphatidylethanolamine, while CRP interacts with phosphorylcholine, both of which are exposed on apoptotic cells. Moreover, SAP and CRP bind to many other ligands, including chromatin and histones, and as such SAP is the major DNA and chromatin binding protein in serum. SAP also interacts with many matrix components including laminin, type IV collagen, fibronectin, and proteoglycans. This means that SAP in contrast with CRP is not only a normal serum protein but also a normal matrix constituent.

All pentraxin-ligand interactions are strongly dependent on Ca^{2+} ions. Importantly, the pentraxins can only interact with early apoptotic cells through phospholipid interactions, unless chromatin structures are exposed at the cell membrane as well.[4] However, in late apoptotic cells the pentraxins may also interact directly with intracellular or free chromatin, and this seems to be more important in their interaction with apoptotic and possibly necrotic cells.[5] After having bound to their ligand, all pentraxins will bind complement C1q, although the mechanism of binding differs between CRP and SAP (Ca^{2+} dependent via the collagen-rich region of C1q), and PTX3 (Ca^{2+} independent via the globular head of C1q). This may have functional consequences for subsequent functions of complement, as well as for the interaction of apoptotic cells with phagocytes.[6]

PHAGOCYTOSIS AND CLEARANCE OF APOPTOTIC CELLS

After opsonisation, the apoptotic cell will interact with phagocytic cells having receptors for the respective opsonising proteins or surface phospholipids. These receptors include the complement receptors, IgG Fcγ receptors, a phosphatidylserine receptor, the vitronectin receptor, a scavenger receptor, and several others. The complement receptors, especially, have a dominant role, and many in vitro systems testing for phagocytosis of apoptotic cells completely depend on the presence of complement.[7]

A major feature of phagocytic clearance of apoptotic cells is that this process leads to suppression of inflammatory reactions, in contrast with the phagocytosis of immune complexes or necrotic cells. A disturbed clearance of apoptotic cells, resulting from defects in the apoptotic process itself, in the process of opsonisation, in the process of binding to and subsequent clearance by phagocytes, or by factors such as autoantibodies interfering with these processes, will lead to a sustained presence of apoptotic cells.

Deficiencies in one or more of these processes have a potential role in SLE. Many animal models for SLE can be generated by interfering with one of the processes in apoptosis.[8] C1q deficient mice develop autoantibodies to nuclear antigens as well as glomerulonephritis.[9] A deficiency in pentraxin production, as exemplified by SAP deficiency, also leads to SLE-like autoimmunity.[10] Strain differences also play a major part in these models, implicating the

influence of other genetically determined factors as well.[11]

APOPTOSIS RELATED AUTOANTIBODIES IN SLE

The sustained presence of apoptotic cells and exposure of intracellular or nuclear antigens that are modified by the process of apoptosis may lead to the production of autoantibodies to these antigens in susceptible people or mouse strains. Autoantibodies to nuclear antigens, especially antibodies to chromatin or nucleosomes including antibodies to double stranded DNA, are most characteristic for SLE. After antigen binding, immune complexes are formed that can be deposited in the kidney by binding to the glomerular basement membrane, leading to glomerulonephritis.

Apart from autoantibodies to the apoptotic structures itself, the presence of autoantibodies to the opsonins of these apoptotic structures like C1q, β_2-glycoprotein I, annexin V, and CRP, have also been described in SLE.[12–15] These autoantibodies may have clinical significance. In this issue of the *Annals* Zandman-Goddard *et al* describe the presence of autoantibodies to the pentraxin SAP in SLE.[16]

AUTOANTIBODIES TO SAP IN SLE

Zandman-Goddard *et al* show that anti-SAP is present in 145/328 (44%) patients with SLE tested and that these autoantibodies have higher titres and are more common in patients with active disease. Although the frequency varied in the four patient populations studied between 22% and 69%, this seems to be a rather high frequency in consecutively sampled patients. Because SAP is a constitutive serum pentraxin and because it has such an important role in the opsonisation of (late) apoptotic cells, it is certainly worthwhile studying these findings further. Many questions will have to be answered.

Firstly, we will need more proof that anti-SAP is really anti-SAP. Analyses of autoantibodies to proteins that strongly interact with nucleosomes and immunoglobulins are notorious for their many pitfalls, as we have learnt from the history of anti-C1q. For example, it has been shown that serum samples from patients with SLE, especially during active disease, may contain nucleosomes complexed with C1q and immunoglobulins that can bind to solid phase coated SAP, leading to false positive results for anti-SAP testing. In addition, studies have to deal with the antigenic specificity: do the antibodies bind to monomeric and/or pentameric SAP, is this binding Ca^{2+} and conformation dependent, is there a difference in binding between native SAP and ligand bound SAP?

Next, one wonders whether anti-SAP production may be the result of the phenomenon of antigen spreading. Most autoantibodies in SLE target proteins that are chemically or structurally modified during the process of apoptosis, so one may assume that native SAP is not the original target antigen. Knowledge of the true antigen certainly also has an impact on anti-SAP testing.

Because mouse models for SLE have taught us so much about the aetiopathogenesis of SLE, it will be interesting to see whether anti-SAP can also be found in these animals. This will enable us to study the ultimate question: does anti-SAP have any clinical relevance? Autoantibodies to SAP, depending on whether they bind native SAP or ligand-bound SAP, may interfere with the opsonising capacity of SAP or the subsequent functions of opsonisation, or both. Anti-SAP may either lead to decreased opsonisation of apoptotic cells and to subsequent increased ''survival'' of apoptotic cells, or it may lead to increased binding of immunoglobulins and activated complement to apoptotic cells and subsequent induction and amplification of inflammation.

In the study of Zandman-Goddard *et al* anti-SAP is found in patients with active SLE, but not in patients with inactive disease, either studied cross sectionally, or longitudinally during treatment in seven patients. This implicates anti-SAP as a marker of disease activity and, as stated by the authors, a prognostic marker. We will have to await further studies to see whether anti-SAP levels are related to disease activity in general, or to specific disease manifestations such as renal or skin disease.

Ann Rheum Dis 2005;**64**:1669–1670.
doi: 10.1136/ard.2005.043505

Authors' affiliations
P C Limburg, M Bijl, Department of Rheumatology and Clinical Immunology, University Medical Centre Groningen, The Netherlands

Correspondence to: Professor P C Limburg, Department of Rheumatology and Clinical Immunology, University Medical Centre Groningen, University of Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands; p.c.limburg@lc.umcg.nl

Accepted 3 October 2005

REFERENCES

1 **Casciola-Rosen LA**, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J Exp Med* 1994;**179**:1317–30.
2 **Manderson AP**, Botto M, Walport MJ. The role of complement in the development of systemic lupus erythematosus. *Annu Rev Immunol* 2004;**22**:431–56.
3 **Garlanda C**, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol* 2005;**23**:337–66.
4 **Familian A**, Zwart B, Huisman HG, Rensink I, Roem D, Hordijk PL, *et al*. Chromatin-independent binding of serum amyloid P component to apoptotic cells. *J Immunol* 2001;**167**:647–54.
5 **Bijl M**, Horst G, Bijzet J, Bootsma H, Limburg PC, Kallenberg CGM. Serum amyloid P component binds to late apoptotic cells and mediates their uptake by monocyte-derived macrophages. *Arthritis Rheum* 2003;**48**:248–54.
6 **Van Rossum AP**, Fazzini F, Limburg PC, Manfredi AA, Rovere-Querini P, Mantovani, et al. The prototypic tissue pentraxin PTX3, in contrast to the short pentraxin serum amyloid P, inhibits phagocytosis of late apoptotic neutrophils by macrophages. *Arthritis Rheum* 2004;**50**:2667–74.
7 **Hart SP**, Smith JR, Dransfield I. Phagocytosis of opsonized apoptotic cells: roles for 'old-fashioned' receptors for antibody and complement. *Clin Exp Immunol* 2004;**135**:181–5.
8 **Gaipl US**, Brunner J, Beyer TD, Voll RE, Kalden JR, Herrmann M. Disposal of dying cells: a balancing act between infection and autoimmunity. *Arthritis Rheum* 2003;**48**:6–11.
9 **Botto M**, Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, *et al*. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat Genet* 1998;**19**:56–9.
10 **Bickerstaff MCM**, Botto M, Hutchinson WL, Herbert J, Tennent GA, Bybee A, *et al*. Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. *Nat Med* 1999;**5**:694–7.
11 **Gillmore JD**, Hutchinson WL, Herbert J, Bybee A, Mitchell A, Hasserjian RP, *et al*. Autoimmunity and glomerulonephritis in mice with targeted deletion of the serum amyloid P component gene: SAP deficiency or strain combination? *Immunology* 2004;**112**:255–64.
12 **Trouw LA**, Groeneveld TWL, Seelen MA, Duijs JMGJ, Bajema IM, Prins FA, *et al*. Anti-C1q autoantibodies deposit in glomeruli but are only pathogenic in combination with glomerular C1q-containing immune complexes. *J Clin Invest* 2004;**114**:679–88.
13 **Marai I**, Tincani A, Balestieri G, Shoenfeld Y. Anticardiolipin and anti-beta-2-glycoprotein I antibodies. *Autoimmunity* 2005;**38**:33–8.
14 **Kaburaki J**, Kuwana M, Yamamoto M, Kawi S, Ikeda Y. Clinical significance of anti-annexin V antibodies in patients with systemic lupus erythematosus. *Am J Hematol* 1997;**54**:209–13.
15 **Sjöwall C**, Eriksson P, Almer S, Skogh T. Autoantibodies to C-reactive protein is a common finding in SLE, but not in primary Sjögren's syndrome, rheumatoid arthritis, or inflammatory bowel syndrome. *J Autoimmun* 2002;**19**:155–60.
16 **Zandman-Goddard G**, Blank M, Langevitz P, Slutsky L, Pras M, Levy Y, *et al*. Anti-serum amyloid component P antibodies in patients with systemic lupus erythematosus correlate with disease activity. *Ann Rheum Dis* 2005;**64**:1698–1702.