

Autoantigens in systemic autoimmunity: critical partner in pathogenesis

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Understanding the mechanisms of human autoimmune rheumatic diseases presents a major challenge, due to marked complexity involving multiple domains, including genetics, environment and kinetics. In spite of this, the immune response in each of these diseases is largely specific, with distinct autoantibodies associated with different disease phenotypes. Defining the basis of such specificity will provide important insights into disease mechanism. Accumulating data suggest an interesting paradigm for antigen selection in autoimmunity, in which target tissue and immune effector pathways form a mutually reinforcing partnership. In this model, distinct autoantibody patterns in autoimmunity may be

viewed as the integrated, amplified output of several interacting systems, including: (i) the specific target tissue, (ii) the immune effector pathways that modify antigen structure and cause tissue damage and dysfunction, and (iii) the homeostatic pathways activated in response to damage (e.g. regeneration/differentiation/cytokine effects). As unique antigen expression and structure may occur exclusively under these amplifying circumstances, it is useful to view the molecules targeted as ‘neo-antigens’, that is, antigens expressed under specific conditions, rather than ubiquitously. This model adds an important new dynamic element to selection of antigen targets in autoimmunity, and suggests that the amplifying loop will only be identified by studying the diseased target tissue *in vivo*.

Keywords: autoantibody, autoantigen, cell death, repair.

Defining autoantigens – the methodology followed influences the outcome

A major quantum in the understanding of autoimmune rheumatic diseases came in 1948, when Hargraves initially described the lupus erythematosus (LE) cell in the bone marrow (BM) of patients with systemic lupus erythematosus (SLE)[1]. This initial observation suggested several paths forward – one of which focused on defining the mechanisms underlying the formation of LE cells *in vitro*, by identifying the necessary components (bait blood cells, phagocytic cells, immunoglobulins and fresh complement). These latter studies (initially pursued by Holman and Kunkel) demonstrated autoantibodies against nuclear

components as a prominent target in systemic autoimmune diseases [2]. The targets of these antibodies were further elucidated over the next several decades in various cell lines *in vitro*, using a variety of assays including immunofluorescence, immunodiffusion and immunoprecipitation [3, 4]. The targets of autoantibodies in SLE and similar systemic autoimmune processes defined in this way turned out to be ubiquitously expressed molecules [4], with distinct patterns of antibody reactivity observed in different autoimmune rheumatic phenotypes [5]. Thus, SLE was characterized by the targeting of dsDNA/nucleosomes [6–9] and splicing ribonucleoproteins [10]; scleroderma by targeting of topoisomerase-1 and centromere proteins [11–13] and myositis by targeting of

aminoacyl tRNA synthetases [14–16]. Of note, autoantigens recognized by rheumatic disease sera were defined in human cell lines available in laboratories which provided a convenient source of autoantigen. The universe of possible autoantigens that could be discovered under these circumstances was therefore limited to those antigens expressed in these cells (Fig. 1). If there were other antigens present in the *in vivo* target of the immune response, these could not be discovered.

Tissue-specific autoimmune diseases provide an excellent exemplar. Myasthenia gravis (MG) is an autoimmune disease where components of the neuromuscular end plate are targeted by the immune system [17]. Some of the molecular components at the neuromuscular end-plate are also found in other nucleated cells. For example, titin is a large myofibrillar protein (>500 kDa) found in 40–70% of myasthenia gravis patients [18, 19]. Titin also plays a role in mitosis and is expressed in various cell lines [19, 20]. Thus if cell lines had been used for initial detection of autoantibodies in MG, titin would have been a prominent antigen identified. This specificity provides little insight however into disease mechanism; such understanding really came from the discovery that subunits of the nicotinic acetylcholine receptor are targeted in MG, and that such antibodies are of pathogenic significance [20].

Thus, whilst the initial path for discovery of autoantigens in systemic autoimmune diseases led to a group of molecules expressed broadly in different cell types, both the cell type and the physiological state of the actual target cell in various systemic autoimmune diseases may await elucidation.

Dying cells are a source of clustered and modified autoantigens

To begin to define physiological states of the target cell which may drive autoimmunity, several important tools are available [21]: (i) sera from well-characterized patient phenotypes can be used to define unique patterns of autoantibody reactivity against specific cell types and states, and (ii) cells can be perturbed with

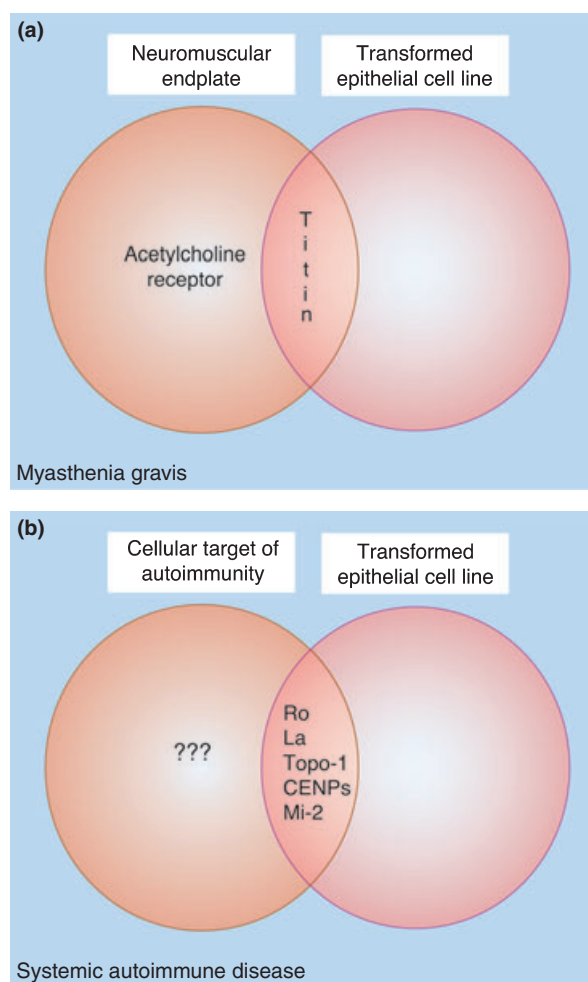


Fig. 1 Screening for autoantibodies: specific versus shared autoantigens. With tissue-specific autoimmune diseases, autoantigens expressed uniquely in the target tissue are demonstrable, and may be of pathogenic significance. For example, when serum from myasthenia gravis is screened against an epithelial cell line, high titre autoantibodies to titin can be demonstrated (a). Titin is a protein enriched at the neuromuscular junction, but is also more widely expressed, as it participates in mitotic pathways. Whilst the neuromuscular endplate expresses both the acetylcholine receptor and titin, HeLa cells express only titin. Screening for autoantibodies against HeLa cells therefore would detect just titin, and the centrally important antibodies to the acetylcholine receptor would not be defined. For systemic autoimmune diseases, the majority of autoantibodies have been defined using transformed epithelial cell lines, making it likely that only the shared group of autoantigens have been defined to date (b). Phenotype-specific autoantigens expressed uniquely in the target tissue under perturbed conditions may remain to be discovered. AChR, acetylcholine receptor.

various environmental stimuli which are associated with disease flare. For example, heightened photosensitivity was recognized early as a feature of SLE, with sunlight exposure potentially causing a flare of both skin and systemic disease [22]. About 15 years ago, we therefore examined the effects of UVB irradiation on distribution and structure of lupus autoantigens [22, 23]. These studies showed that various lupus autoantigens are strikingly clustered and concentrated in surface blebs on apoptotic cells. These antigens share nothing in common in the control setting, suggesting that modifications of autoantigen structure and distribution in apoptotic cells might play a role in the selection of autoantigens in SLE. Indeed, additional studies from our group and numerous others over the next decade demonstrated that these autoantigens are in fact susceptible to various post-translational modifications, which influence their immunogenicity [24]. For example, numerous autoantigens are cleaved by various apoptotic proteases [25–31] and several autoantigens are phosphorylated or dephosphorylated during apoptosis [32–34]. Additionally, multiple nucleic acid/nucleosome modifications occur during apoptosis, e.g. histone is modified by acetylation [35] and RNA by cleavage or other changes [36, 37]. In several instances, the autoantibodies preferentially recognize the modified form of the antigens [38–41].

In general, apoptotic cells are rapidly cleared *in vivo*, and are associated with anti-inflammatory consequences (e.g. secretion of IL-10 and TGF- β by the phagocytizing macrophage) [42–44]. It is therefore unlikely that modified autoantigens in apoptotic cells access antigen-presenting cells in a pro-immune setting under normal circumstances (Fig. 2). It is noteworthy that numerous studies have demonstrated that abnormalities of apoptotic cell clearance may be associated with systemic autoimmunity, suggesting that delayed apoptotic cell clearance might allow post-translationally modified antigens to be seen in a pro-immune context. In this regard, human and mouse deficiency in C1q is associated with accumulation of apoptotic material in various tissue microenvironments, and the initiation of autoimmunity with a lupus-like phenotype [45]. Deficiency of MFG-E8 (milk fat globule epidermal growth factor like-8) is also strikingly associated with

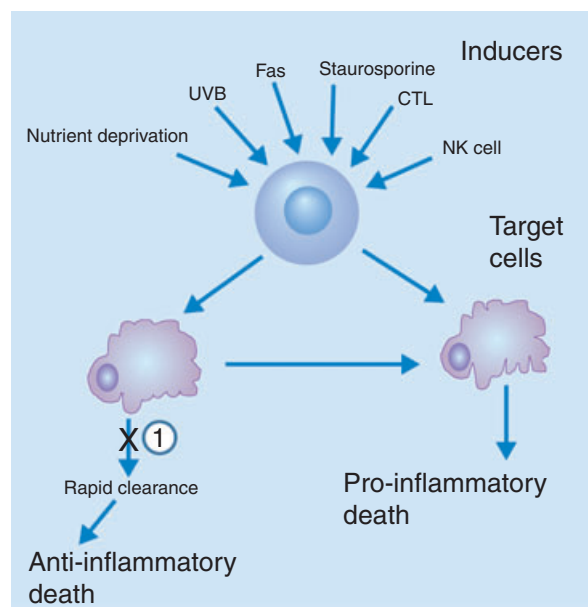


Fig. 2 Rapid, efficient clearance of dying cells is required to prevent emergence of autoimmunity. Numerous upstream inducers (activating extrinsic and intrinsic pathways) can initiate apoptotic cell death. Under normal homeostatic circumstances, apoptotic cells are rapidly cleared by phagocytic cells, resulting in the secretion of anti-inflammatory cytokines and the induction of T-cell tolerance. These pathways provide a major barrier to the induction of autoimmunity. Several abnormalities may allow this barrier to be overcome: (i) impaired clearance of apoptotic cells, allowing dying cells to persist for longer times and undergo changes that make them immunogenic; and (ii) different types of death (e.g. cytotoxic lymphocyte granule-induced death) in which the structure of many self-antigens is modified to forms not previously tolerized by the host. Such pro-inflammatory forms of cell death may be important initiators of autoimmunity.

decreased clearance of apoptotic cells in lymph nodes by tingible-body macrophages, and development of systemic autoimmunity with a lupus-like phenotype [46, 47]. It is also noteworthy that apoptotic cells can drive autoantibody responses in animal models of systemic autoimmunity [48].

Different forms of cell death may be relevant in different systemic autoimmune processes

Although numerous inducers of apoptosis exist, in most cases, the final phenotype of death is very similar in all these circumstances, and the immune

consequences are similarly anti-inflammatory. Other than abnormalities in clearance mechanisms, are there additional mechanisms which might increase the propensity of apoptotic cells to induce an immune response (Fig. 2). Whilst increased numbers of uncleared apoptotic cells can be a feature in SLE tissues, this is not observed in other rheumatic phenotypes (e.g. Sjogren's syndrome and myositis). Interestingly however, the cytotoxic lymphocyte granule pathway is highly expressed in various autoimmune pathologies, with clear evidence of active cytotoxic lymphocytes in several conditions, including autoimmune myopathies, SLE, rheumatoid arthritis (RA) and Sjogren's syndrome [49–52]. In both SLE and RA, there is some evidence that activity of these cytotoxicity pathways is associated with disease activity. Although the cytotoxic lymphocyte granule pathway can generate a typically apoptotic form of death with activation of caspases, there are clearly caspase-independent pathways activated by cytolytic cell granule proteases (granzymes) that are not observed during these more homeostatic forms of cell death [53, 54]. Additionally, whilst classic forms of apoptosis generally occur in homeostatic settings, the cytotoxic lymphocyte granule pathway is generally activated against infected or transformed cells, typically pro-immune settings. Interestingly, myositis autoantigens are almost all susceptible to cleavage by cytotoxic lymphocyte granule proteases, generating unique fragments not observed under other circumstances [55, 56]. Activated cytotoxic lymphocytes which have the capacity to generate novel autoantigen fragments, have been demonstrated at increased levels in patients with SLE, where their numbers correlate well with disease activity [52, 57]. Although some autoantibodies from rheumatic disease patients preferentially recognize cleaved over uncleaved forms of the antigen [41], this is an uncommon feature. The effect of cleavage of autoantigens on their immunogenicity for T cells remains unknown, but will be very important to define.

Thus, novel pro-immune forms of cell death, generating unique structure of autoantigens not generated during homeostatic forms of cell death, may be an important focus of the immune system in systemic autoimmunity.

Which cells in tissue provide the antigen stimulus in autoimmunity? The roles of homeostatic and inflammatory processes in the target tissue

Distinct groups of autoantigens are targeted in various systemic rheumatic processes, though the origin of this specificity remains unknown. Although it has been largely assumed that autoantigens targeted in various rheumatic autoimmune diseases are ubiquitously expressed, the evidence for this *in vivo* is minimal. We therefore set out to define the expression patterns of phenotype-specific autoantigens *in vivo* [56]. Our initial work focused on the expression of myositis-specific autoantigens in normal versus myositis muscle. Our findings were remarkable and quite unexpected. We found myositis autoantigen levels in normal muscle to be low, but high in myositis muscle. Furthermore, within myositis muscle, the highest levels of autoantigens were found in muscle cells that had features of regeneration. Interestingly, the dermatomyositis (DM) autoantigen, Mi-2, was expressed at particularly high levels in DM muscle, highlighting the congruence between antigen expression patterns in the target tissue and targets of the immune response in that disease [56]. These data strongly suggest that healthy muscle cells are not the targets of autoimmunity in myositis, but rather, that regenerating muscle – responding to various injuries – is the target of ongoing immune damage in this disease.

Another prominent autoantigen in myositis is Ro-52, an interferon inducible protein [58, 59]. The interferon gene expression signature is prominent in DM muscle and blood [60, 61]. We have recently demonstrated that Ro52 and other interferon-inducible antigen expression is increased in muscle biopsies from dermatomyositis patients (J. Hall, L. Casciola-Rosen, A. Mammen and A. Rosen, unpublished data), again indicating that multiple pathways regulate autoantigen expression in the target tissue, and might sculpt the disease-specific immune response.

Autoimmunity: autoantigens versus neoantigens

The classic view of autoimmunity is that the immune system targets autoantigens expressed in self tissues.

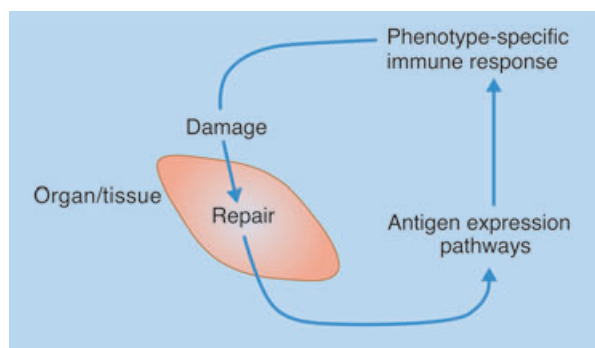


Fig. 3 A mutually reinforcing cycle drives antigen selection and immune amplification in autoimmune diseases. Normal muscle expresses low levels of myositis autoantigens, whereas these are expressed at high levels in regenerating muscle. In the setting of a cytotoxic lymphocyte response against these antigens (e.g. either induced locally during tissue damage or distantly during an anti-tumour response), tissue damage induces healing, with regenerating cells expressing high levels of the same antigens that drive the cytotoxic lymphocyte response. A cycle is therefore set up, in which the immune response drives damage, which stimulates antigen expression, and this in turn targets additional damage onto the regenerating cells. Of note, both the target tissue and the immune system contribute to this feedforward cycle, making it likely that impacting either component will have therapeutic benefit.

Although the assumption has been that such antigens are expressed under all circumstances, the data reviewed above strongly suggest that this assumption may be too simple and static. Indeed, they suggest an interesting paradigm for antigen selection in autoimmunity, in which target tissue and immune effector pathways form a mutually reinforcing pair (Fig. 3). Depending on the specific target tissue, the pathways activated (e.g. regeneration/differentiation/cytokine exposure), and the immune effector pathways that modify antigen structure, so unique immune targets are selected and amplified. Patterns of autoantibodies in autoimmunity may therefore be viewed as the integrated, amplified output of unique target tissue, specific stimulated gene expression pathways and distinct combination of immune effector forces. As unique antigen expression and structure may occur exclusively under these amplifying circumstances, it is useful to view the molecules targeted as ‘neo-antigens’ – that is, antigens expressed under specific conditions, rather than ubiquitously. This view clearly adds a dynamic element to selection of antigen targets in autoimmunity,

and suggests that the amplifying ‘duo’ will only be identified by studying the diseased target tissue *in vivo*.

Redefining autoantigens – looking for new paths

If one embraces the concept of ‘neoantigen-driven’ immune tissue injury as playing an important role in systemic autoimmune diseases, generating the appropriate source of material for autoantibody screening is paramount. It is interesting to revisit the LE cell with this in mind. This cell occurs *in vivo* in BM from SLE patients, and requires the presence of autoantibodies from patient serum to be generated *in vitro*. Defining the BM cell *in vivo* which is the source of phagocytic material in the LE cell may identify novel autoantigens in SLE, and the cell type that contributes to the ongoing antigen drive in the disease. The recent identification of a granulopoiesis signature in patients with active SLE may provide a starting point for such studies [60]. Such principles may also be applicable to many forms of systemic autoimmunity.

Conflict of interest statement

No conflict of interest was declared.

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