In this issue of the Journal, Casciola-Rosen et al. (1) call attention to a topic that has been of recent interest, the possible association of apoptosis with autoimmunity. Apoptosis, sometimes called programmed cell death, was described by Kerr et al. (2) and Wyllie et al. (3) as a form of cell death characterized by cell shrinkage, nuclear condensation, and surface blebbing not accompanied by inflammatory cell infiltration. In tissues, it affects scattered single cells rather than tracts of contiguous cells, and fragments of apoptotic cells are phagocytosed and digested by resident cells; in epithelial tissue, they are taken up by adjacent epithelial cells. A generally accepted biochemical correlate of apoptosis is the cleavage of chromatin at the internucleosomal linker regions to produce nucleosomal fragments of different lengths which, in agarose gel electrophoresis, appear as DNA ladders (4). In systemic lupus erythematosus (lupus), which is often regarded as a prototypic systemic autoimmune disease, there is a characteristic autoantibody response directed against selected intracellular antigens. Casciola-Rosen et al. show that UV irradiation of cultured human keratinocytes induces changes consistent with apoptosis and that certain clusters of appropriate antigens are grouped together in different apoptotic blebs in these damaged cells.

The skin has long been recognized as an organ that plays an important role in lupus. This is related to the fact that skin rashes are a dominant feature of lupus. Characteristic deposits of immunoglobulin and complement at the dermal-epidermal junction imply the participation of antibodies in its pathogenesis (5). In addition, the disease can manifest clinically de novo after extended exposure to sunlight and ongoing disease might be exacerbated after such exposure. For these reasons, the skin and keratinocytes have been objects of investigation by several groups (6–8). The novelty of the Casciola-Rosen study is the recognition of UV-induced injury to keratinocytes as consistent with apoptosis and the identification of clusters of nuclear and cytoplasmic antigens in different translocated sites. A population of smaller apoptotic blebs contained fragmented endoplasmic reticulum, ribosomes, and a class of ribonucleoproteins called SS-A/Ro, and larger apoptotic blebs contained nucleosomal DNA in addition to ribonucleoproteins.

The prevailing dogma as to how the human organism protects itself from autoimmune reactions comes primarily from studies in the mouse showing that immature self-reactive T cells are deleted or become anergic in the thymus when confronted with self-antigens on thymic presenting cells, including bone marrow–derived dendritic cells, a process that has been called negative selection (9, 10, 11). It appears that one mechanism by which this type of clonal T cell deletion takes place is apoptosis (12, 13). The possible relevance of these observations to autoimmunity was heightened by the report that the MRL/Mp-lpr/lpr mouse, which develops a disease analogous to human lupus, has an abnormality in the Fas gene that mediates apoptosis (14). This abnormality has been determined to result from integration of the retrotransposon, Etn, into the second intron of the Fas gene resulting in diminished Fas expression and presumably leading to diminished or absent Fas-mediated apoptosis (15–17). However, several investigators have reported that lpr mice do have the potential to delete autoreactive T cells (18–20). It has been suggested that in spite of the Fas gene abnormality observed, there is the possibility that low levels of Fas protein might be expressed (15, 17). At present, the importance of the Fas gene in autoimmune murine lupus appears to be unresolved. If it does have a role, it is not a universal feature since abnormalities of Fas mRNA expression have not been detected in NZB/W thymus or spleen (17), another murine model of lupus which is perhaps closer in clinical and serological features to human lupus than the lpr model. Furthermore, the MRL/Mp-+/+ mouse, which is genetically identical to MRL/lpr except for the lpr abnormality, also develops lupus, although later in life.

A striking feature that has emerged from studies to learn more about the structure and function of intranuclear autoantigens is that many of these antigens are involved in important or key biosynthetic functions. Some of these are shown in Table 1. In diseases like lupus, mixed connective tissue disease, scleroderma, and dermatomyositis, autoantigens are involved in fundamental cell functions such as transcription, precursor messenger RNA splicing, DNA replication, cell division, and ribosomal protein synthesis (for a review see reference 21). Why cellular components participating in these important biosynthetic functions are targeted for autoimmune reactions is entirely unclear at the present time. The major autoantigens in these diseases have now been identified (21), and it appears that there might be a preferential autoantibody response to biosynthetic nuclear and cytoplasmic components and a paucity of antibody responses to structural and membrane components.

Many investigators have been interested in defining the antigenic determinants or epitopes that are recognized by autoantibodies. An emerging consensus from these studies is that many autoepitopes are not continuous primary sequence peptides but are conformation dependent and made up of...
**Table 1. Some Autoantigens Involved in Cellular Biosynthetic Functions**

| Disease                        | Identity and structure of antigen                                                                 | Function of antigen                  |
|-------------------------------|--------------------------------------------------------------------------------------------------|--------------------------------------|
| Lupus                         | Sm -common proteins of small nuclear ribonucleoprotein particles (snRNP), U1,U2,U4-U6 snRNPs      | Splicing of precursor messenger RNA  |
| Lupus                         | PCNA-Auxiliary protein of DNA polymerase delta                                                   | DNA replication-leading strand synthesis |
| Lupus and mixed connective tissue disease | U1 snRNP-specific proteins of U1 RNP particle                                                      | Splicing of pre-mRNA                 |
| Lupus and scleroderma         | NOR-90-Nucleolus organizer region proteins                                                        | RNA pol I transcription              |
| Scleroderma                   | Scl-70-DNA topoisomerase I                                                                      | Relaxation of supercoiled DNA        |
| Scleroderma (CREST*)          | Centromere-associated proteins of 17,80, and 140 kD                                              | Spindle attachment in cell mitosis   |
| Dermatomyositis               | Transfer RNA (tRNA^{his,thr,ala}) synthetases                                                     | Ribosomal protein synthesis          |

* CREST is the acronym for a subset of scleroderma characterized by Calcinosis, Raynaud's phenomenon, Esophageal dysmotility, Sclerodactyly, and Telangiectasia.

Discontinuous sequences either as a result of protein folding or of protein–protein interactions (22-25). A case in point involved studies on proliferating cell nuclear antigen (PCNA) where it was shown that lupus autoantibodies were totally nonreactive with continuous sequence peptides of PCNA in contrast to experimentally induced murine monoclonal antibodies which reacted strongly with these peptides (26). The significance of the observation that human autoantibodies frequently recognize only conformation-dependent peptides was reinforced by the findings that human autoantibodies were capable of inhibiting the known functions of autoantigens such as splicing by snRNP particles, DNA replication (by PCNA), and ribosomal protein synthesis (by tRNA synthetases). In the case of PCNA and tRNA^{his} synthetase, experimental monoclonal antibodies recognizing continuous sequences were ineffective (27, 28). The cumulative data strongly suggest that many autoepitopes involve the active sites or functional domains of these intracellular particles and it might be expected that these regions would be evolutionarily conserved. This notion is already implicit in routine testing when detection of autoantibodies to nuclear and cytoplasmic antigens can be ascertained in immunofluorescence, using tissue or cell culture substrates ranging widely across species from mammalian to avian, amphibian, and sometimes to plant kingdoms. The highly conserved nature of epitopes recognized by autoantibodies is illustrated in a study depicted in Table 2 where reactivity of a human autoantibody to SS-B/La, a nuclear protein of 48 kD involved in RNA polymerase III transcript maturation, is compared with reactivity of several experimentally induced murine monoclonal antibodies to purified bovine antigen (29). Murine antibodies were nonreactive with antigen in species more closely related to itself whereas human antibody was pan-reactive.

The central question that has always arisen is why autoantibodies are mounted against ubiquitous self-antigens that are functionally important. Perhaps some clues to this issue are coming from studies on p53, a tumor suppressor gene that has been called the 1993 molecule of the year (30). The p53 molecule has a role in transcription and in cell cycle control so that in these properties it is much like lupus and other autoimmune antigens. About 10% of patients with breast cancer have circulating antibodies to p53 protein (31). In these patients, there were missense mutations in exons 5 and 6 of the gene resulting in p53 overexpression, whereas no antibodies were detected in patients with low level p53 expression. Moreover, tumors in patients with antibodies contained complexes between p53 and a 70-kD heat shock protein. It was suggested that the 70-kD protein might be involved in antigenic presentation of p53. It was also noteworthy that antibodies reacted with wild type p53 as well as mutant p53 proteins. Antibodies to p53 have also been detected in about 13% of patients with lung cancer, and it was also shown that these patients also had missense mutations in certain exons (32). In view of these findings, it would be of interest to
| Species   | Cell line          | Immunofluorescence* | Murine mABs | Human serum |
|-----------|--------------------|---------------------|-------------|-------------|
| Human     | Hep-2, larynx      | +                   | +           | +           |
| Human     | HeLa, cervix       | +                   | +           | +           |
| Human     | Raji, Burkitt, lymphoma | +          | +           | +           |
| Monkey    | Vero, kidney       | +                   | +           | +           |
| Rabbit    | R9ab, lung         | +                   | +           | +           |
| Bovine    | MDBK, kidney       | +                   | +           | +           |
| Hamster   | BHK-21, kidney     |                     |             |             |
| Rat       | 6m2, kidney        |                     |             |             |
| Mouse     | 3T3 fibroblast     |                     |             |             |
| Rat kangaroo | PtK2, kidney   |                     |             |             |

* Immunofluorescence performed on cell lines to detect nuclear fluorescence of anti-SS-B/La specificity (29).

determine whether these abnormalities might be present in lupus and other systemic autoimmune diseases.

Many questions remain to be explored in order to elucidate mechanisms related to human autoimmune diseases, and the stage may be set for significant advances since some of the current knowledge summarized above points to certain directions of investigation. The work of Casciola-Rosen et al. suggests that apoptosis might be a mechanism inducing the clustering of potentially immunogenic cellular components, a process which might explain why antibodies in a particular disease are directed at multiple antigens. The outcome of further studies to test this notion would clearly be of interest.

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