

## Editorial

# The effect of UVB on lupus skin: new light on the role of apoptosis in the pathogenesis of autoimmunity

Apoptosis is a major form of programmed cell death and occurs in both physiological and pathological conditions. With apoptosis, cells display distinct morphological and biochemical features that allow their identification and enumeration both *in vivo* and *in vitro*. Prominent changes in cells undergoing this process are chromatin condensation, DNA fragmentation, membrane blebbing and externalization of phosphatidylserine of the cell membrane [1]. Once apoptosis is initiated, alterations in membrane structure mark a cell for recognition by macrophages that can engulf the dying cell for safe disposal. Indeed, the phagocytosis of dead cells is one of the critical functions of the immune system in preventing inadvertent immune activation by proinflammatory cellular material arising during death. Given the potential impact of dying cells and their products on the immune system, disturbances of the cell death and clearance processes have been postulated to occur in a variety of human diseases, including autoimmune syndromes such as systemic lupus erythematosus (SLE).

In this issue of *Rheumatology*, Reefman and co-workers explore possible abnormalities of cell death in SLE by investigating the frequency of apoptotic cells in the skin resulting from exposure of defined areas to UVB light. Ultraviolet radiation, in particular UVB with a short wavelength range (between 290 and 320 nm), represents an important environmental factor that can trigger a variety of deleterious responses in humans. The intracellular processes induced by UVB are complex, cell injury and death being mediated by various mechanisms, including DNA damage, the production of reactive oxygen species and the activation of cell death receptors [2]. In the skin, UVB is well characterized as an inducer of cutaneous damage, causing the accumulation of apoptotic keratinocytes at the dermo-epidermal junction. Given the toxic products induced by UVB exposure, apoptosis may represent part of a protective mechanism to remove damaged cells and reduce the potential risk to the system from spillage of their contents.

In SLE, the outcome of exposure to UVB radiation is photosensitivity and the development of photolesions in the skin, as observed typically in cutaneous lupus. The amount of energy needed for UVB to provoke cutaneous changes is usually referred to as the minimal erythemal dose (MED). Since photosensitivity is one of the clinical hallmarks of lupus, it may not be surprising that the SLE patients in the study of Reefman *et al.* had a lower threshold than normal subjects for the development of an UVB-induced erythema. This observation confirms data from other studies [3], although the finding that UVB-induced erythema can be associated with an increased frequency of apoptotic cells (detected by light microscopy or immunochemical staining for cleaved caspase 3) is novel. Importantly, in the context of models for the pathogenesis of SLE, it is notable that the number of apoptotic cells and the amount of pycnotic nuclear debris observed histologically in the skin of patients and healthy volunteers were similar after 24 h. The development of erythema thus appears to be directly linked to the number of apoptotic cells, but the extent to which cells die appears to be independent of the presence of underlying autoimmunity. These data thus suggest that keratinocytes of SLE patients have normal sensitivity to UVB-mediated apoptosis, at least under the conditions of the present study.

Studies of several mouse models of SLE have suggested a direct link between apoptosis and the development of lupus. For example, defects involving the induction of apoptosis through the Fas/FasL system [4], overexpression of the anti-apoptotic molecule Bcl-2 [5] or heterozygosity for *Pten* (a tumour-suppressor gene involved in the induction of apoptosis) [6] are all associated with an increased number of autoreactive cells leading to lymphoproliferation and autoimmunity. T and B lymphocytes from these mice showed highly increased populations positive for several activation markers as well as hypergammaglobulinaemia with autoantibodies against nuclear antigens. Moreover, the kidneys of these mice showed glomerular abnormalities due to deposition of immune complexes. However, the effects of UVB irradiation have not been extensively investigated in lupus mouse models with respect to the induction of apoptosis [7] or the potentiation of autoimmunity.

The evidence for disturbed apoptosis in human lupus, on the other hand, is less clear. Increased levels of circulating apoptotic cells have been found in SLE patients [8–10], although levels did not correlate with disease activity in these studies. Data on apoptotic keratinocytes in the skin lesions of SLE patients are controversial with respect to both the induction of apoptosis and correlation with autoantibodies [11–13]. These differences could be due to the influence of various medication schemes or differences in the patient population studied.

While Reefman *et al.* have shown a similarity in the frequency of apoptotic cells in patients with SLE and normal subjects treated with UVB, these findings do not exclude an important role of defective apoptosis in the pathogenesis of lupus. Apoptosis is a highly heterogeneous process that may vary in time-course and biochemical features depending on cell type, location and trigger. In the context of the current experiments, it is possible that the number of apoptotic cells or the amount of nuclear debris would differ between patients and controls at later time-points of investigation. Similarly, it is possible that differences between patients and normal subjects would be more pronounced if UVB were applied repeatedly or in different concentrations over a certain period instead of in one or two single doses. Moreover, the duration of disease and activity of cutaneous lesions might also influence the extent of apoptosis following UVB exposure. The findings, while intriguing in terms of existing models for SLE, must nevertheless be interpreted with caution, given the heterogeneity in the dynamics and mechanisms of cell death processes.

In addition to altered induction of apoptosis, the removal of apoptotic cells has attracted interest in SLE as an important element in the pathogenesis of the disease [14–17]. The clearance of potentially autoantigenic material or debris created by dying cells is a highly regulated process that may actively inhibit the initiation of inflammation and immune responses. Cells undergoing apoptosis are removed rapidly through non-inflammatory engulfment by macrophages or even non-professional phagocytes such as fibroblasts, endothelial cells, or vascular smooth muscle cells [18]. The clearance process involves a host of cellular and humoral elements to promote phagocytosis prior to the transition of dying cells to secondary necrosis, a proinflammatory state, or access of apoptotic cells to dendritic cells, where uptake would lead to antigen presentation.

Several defects are known in humans with respect to the clearance of dying cells and cellular material. In the context of SLE, in particular, relative failure of the acute-phase CRP response [19], reduced activity of DNase I [20, 21] and complement defects [22] could all contribute to impaired clearance of apoptotic cells and the accumulation of cells in late apoptosis, a phase also termed 'secondary necrosis'. While the cellular events in the late stages of the death processes are not well defined, they may involve leakage of proinflammatory molecules into the external milieu; the generation of new moieties from proteolytic digestion or the action of reactive oxygen species may also occur. A further build-up of secondarily necrotic cells may occur when the apoptotic load exceeds the local capacity for phagocytes to mediate clearance, a situation that could reflect either increased death or failed clearance. The time-course for these events is speculative and it is possible that the experiments of Reefman *et al.* were not long enough to detect defects in the phagocytosis of apoptotic keratinocytes, a phenomenon that might explain the increased rate of apoptotic cells in established skin lesions in SLE patients.

More than 10 years ago, in seminal experiments Casciola-Rosen *et al.* [23] showed that keratinocytes undergoing apoptosis after exposure to UV light cluster a variety of macromolecules in blebs on the cell surface. Importantly, these small blebs contained the major autoantigens recognized by lupus sera. From these studies, it can be hypothesized that the persistence of these blebs due to impaired removal of apoptotic cells could lead to the release of antigenic structures into the surrounding tissue or circulation. Once released, these structures might induce an immune response culminating in the production of autoantibodies. In their study, Reefman and co-workers did not find a correlation between MED values and the presence of autoantibodies in the serum. Other investigators, however, have shown such a correlation [13], in particular by showing a positive association between anti-Ro (SS-A)/anti-La (SS-B) autoantibodies and a pathological photoprovocation. This issue awaits further experimentation under other conditions.

While the study of Reefman *et al.* did not show increased apoptosis following UVB exposure in patients with SLE, it is nevertheless important in providing an *in vivo* test for an important hypothesis. However, these findings must be viewed tentatively, given the complexity of the death process and the multitude of ways in which cells can succumb. Sunlight remains hazardous to patients and it is still too early to conclude that UVB-induced apoptosis in SLE is intact and that UVB does not play a role in inducing cell death and injury that provokes autoreactivity.

The skin is a unique system for study since it is a very accessible site and allows experiments not possible with other organs. It will be a challenge to investigators to devise experimental systems to assess death processes in tissues that cannot be readily biopsied or followed after exposure to agents that are too toxic for systemic administration. While the studies of Reefman *et al.* shed some light on an important subject, they provide a reminder that, like life, death is a complicated process and its role in SLE still appears to be not well understood.

The authors have declared no conflicts of interest.

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