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Accelerated In Vitro Apoptosis of Lymphocytes from Patients with Systemic Lupus Erythematosus¹

Woodruff Emlen,² JoAnn Niebur, and Richard Kadera³

Division of Rheumatology, University of Colorado Health Sciences Center, Denver, CO 80262

SLE is a disease characterized by the generation of an immune response to intact nuclear Ags, especially components of the nucleosome, histones and DNA. The process of programmed cell death, or apoptosis, is characterized by cleavage of chromatin into oligonucleosomes and release of these nucleosomes into the extracellular space. To address the question of whether altered apoptosis might provide a source of extracellular nuclear Ags in SLE, we have examined apoptosis of lymphocytes isolated from patients with SLE, patients with rheumatoid arthritis (RA), and normal controls. Apoptosis was measured by three independent methods and confirmed by gel electrophoresis. Freshly isolated lymphocytes (t_0) showed low levels of apoptosis. However, lymphocytes from SLE patients demonstrated a significant increase in the number of apoptotic cells at t_0 compared with normal controls and RA patients. In tissue culture, lymphocytes from all patient groups underwent apoptosis, but the rate of apoptosis of lymphocytes derived from SLE patients was 2.35-fold faster than apoptosis of lymphocytes from normal controls or RA patients. The increased rate of apoptosis could not be accounted for by corticosteroid or cytotoxic medication. There was a significant correlation between SLE disease activity as measured by the systemic lupus activity measure and rate of apoptosis in vitro. The release of intact nucleosomes during apoptosis was measured by ELISA; lymphocytes from SLE patients released increased amounts of nucleosomal material into the extracellular space in direct proportion to the rate of apoptosis. Abnormal apoptosis of lymphocytes in SLE may provide a source of extracellular nuclear Ag to drive the immune response and to allow the formation of immune complexes. The demonstration of altered in vitro apoptosis of lymphocytes derived from SLE patients raises the possibility that abnormalities of apoptosis may contribute to the pathogenesis of SLE. *Journal of Immunology*, 1994, 152: 3685.

SLE is characterized by the production of antibodies to multiple nuclear Ags, including Abs to DNA and histones. Production of these Abs appears to be Ag driven: Antinuclear Abs in SLE are frequently IgG isotype and high avidity (1, 2), and genetic analysis of these antibodies shows somatic mutation (3, 4). Analysis of the fine specificity of these Abs has shown that the epitopes recognized by many antinuclear Abs are on the exposed surfaces of intact nuclear Ags (5, 6). These observations suggest that nuclear Ags must interface with the

immune system to drive the antinuclear Ab response. Thus, both to drive the immune response and to provide Ag for the formation of immune complexes, nuclear Ags must become available in the extracellular space.

A major mechanism by which undigested, intact nuclear Ags are generated and released in vivo is by the process of programmed cell death or apoptosis (10, 11). In contrast to necrosis, in which cell lysis and digestion of cellular contents are early events, apoptosis is characterized by the ordered digestion of nuclear chromatin yielding intact oligonucleosomes (12–14). In tissues, apoptotic cells may be phagocytosed as complete cells (15, 16). However, binding of intact apoptotic cells from solution is inefficient (17), and data indicate that nucleosomes are released into the extracellular milieu during the process of apoptosis (10, 18). One can postulate, therefore, that the process of apoptosis may provide a source of nuclear Ags to drive the autoantibody response and provide Ag in SLE.

Recent reports have suggested that apoptosis may be abnormal in autoimmune disease and may play a role in the induction of autoimmunity (19–21). In the MRL-*lpr*/

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² Address correspondence and reprint requests to Dr. Woodruff Emlen, Division of Rheumatology B-115, University of Colorado Health Sciences Center, 4200 E. 9th Avenue, Denver, CO 80262.

³ Richard Kadera is a medical student at the University of Colorado, Denver, CO.

lpr mouse, a structural defect in the *fas* gene results in decreased apoptosis of peripheral lymphocytes (19, 20) and gradual accumulation of these lymphocytes, resulting in lymphoproliferation. It has been postulated in this model that a failure to peripherally delete T cells by apoptosis results in accumulation of lymphocytes and autoimmunity (21). When lymphocytes from these mice are cultured in vitro, however, they show accelerated apoptosis compared with cells from normal mouse strains (22). To resolve this apparent discrepancy between decreased apoptosis in vivo and accelerated apoptosis in vitro, Van Houten and Budd have postulated that *lpr/lpr* lymphocytes are "primed" for apoptosis, but are prevented from dying in vivo. Once these primed cells are placed in culture ex vivo, they die rapidly as a result of cytokine withdrawal or nonspecific perturbation of the immunologic balance maintained in vivo (22).

In light of the apparent requirement for intact extracellular nuclear Ags to drive the antinuclear Ab response and in light of the reported abnormalities of apoptosis in a murine model of SLE, we have investigated in vitro apoptosis of lymphocytes isolated from SLE patients and compared apoptosis of these cells with apoptosis of lymphocytes from patients with RA⁴ and normal healthy controls. Our data indicate that apoptosis of lymphocytes from SLE patients is accelerated in vitro, similar to the findings reported in MRL-*lpr/lpr* lymphocytes and that, because of this accelerated apoptosis, increased amounts of nucleosomes are released into the extracellular milieu.

Materials and Methods

Human subjects

Patients with SLE and RA by American College of Rheumatology criteria were enrolled from the University of Colorado Health Sciences Center Arthritis Clinic on a serial basis without regard to disease activity. Age and sex-matched controls were obtained from healthy laboratory and secretarial personnel working at the Health Sciences Center. After informed consent was obtained, clinical information was recorded, medications and dosages were recorded, and blood was drawn into heparinized tubes. SLE patients were examined, interviewed, and a disease activity index was obtained according to the method of Liang et al. (systemic lupus activity measure or SLAM) (23).

Cell isolation and culture

Within 60 min after blood drawing, peripheral blood mononuclear cells were isolated by Ficoll-Hypaque gradient as previously described (18) and resuspended in RPMI/5% autologous serum at a concentration of 10^6 /ml. These cells were plated on 24-well plastic tissue culture plates (Falcon 3047, Becton Dickinson, Lincoln Park, NJ) at 37°C for 1 h to allow monocytes to adhere. Nonadherent cells were removed, washed, and resuspended in RPMI/10% FCS at a concentration of 10^6 /ml and cultured in 24-well microtiter plates. FACS analysis of these cells showed that less than 2% of the cells were monocytes. In all patients, cell isolation was complete within 5 h of venipuncture.

Measurement of apoptosis

Aliquots of cells were removed immediately after cell isolation (t_0), and at 24 and 48 h after initiation of culture. Three methods were used to assess apoptosis:

1) Morphologic: Cells were stained with acridine orange (AO) (Sigma) by adding 1 μ l of an AO solution (100 μ g/ml) (24) to 25 μ l of cells. After thorough mixing, cells were fixed by the addition of 20 μ l of 1% paraformaldehyde. Cells were analyzed within 24 h by fluorescence microscopy. Cells were scored as apoptotic if they showed nuclear condensation or fragmentation (24). More than or equal to 200 cells were read per sample point by two to three blinded observers. Interobserver agreement was excellent.

2) Diphenylamine assay: DNA fragmentation was quantitated according to the diphenylamine method of Duke and Cohen (24). Briefly, 3×10^6 lymphocytes were spun at $200 \times g$ for 10 min, and the cell supernatant (S) removed. The cell pellet was then lysed with 0.2% TritonX-100 in Tris-EDTA, followed by centrifugation at $13,000 \times g$ for 10 min to separate fragmented (T, top) from unfragmented (P, pellet) DNA. DNA in each fraction was quantitated by adding diphenylamine reagent (Fisher) (24) and reading the OD at 600 nm after an overnight incubation. Percent of DNA fragmentation was calculated according to the formula $(S + T/S + T + P) \times 100$.

3) FACS analysis: Cells were lysed and stained with propidium iodide (PI) according to the method of Nicoletti et al. (25) and analyzed using an Epics C cell sorter (Coulter Electronics Corp., Hialeah, FL). Because of the fragmentation of chromatin during apoptosis, the nuclei of apoptotic cells take up less PI than the nuclei of normal cells, resulting in a decreased fluorescence signal from apoptotic nuclei. Nuclei were scored as apoptotic if they demonstrated a decrease in PI fluorescence relative to the nuclei of healthy nonapoptotic cells.

To confirm apoptosis, in some experiments, DNA was isolated and analyzed by agarose gel electrophoresis. Cells and nuclei were lysed with (10 mM Tris, pH 8.0) followed by centrifugation for 10 min at 13,000 rpm in a microcentrifuge to separate intact from fragmented DNA (24). The fragmented DNA was digested with protease K and RNase, phenol/chloroform extracted and ethanol precipitated (26). DNA was analyzed on a 1% agarose gel using a 123-bp ladder (GIBCO BRL, Gaithersburg, MD) as a M.W. standard.

Nucleosome ELISA

At varying intervals after initiation of culture, 10^6 cells were removed from culture, spun at low speed (2 min at 3000 rpm in a microfuge), and cell-free supernatants were removed. These supernatants were assayed for nucleosome content in a nucleosome-specific ELISA as previously described (18). Briefly, a mAb reactive with H2A-H2B (27) was bound to 96-well plastic Immulon-2 ELISA plates (Dynatech Labs., Inc., Chantilly, VA) followed by blockade with gelatin. Tissue culture supernatants or isolated calf thymus nucleosomes (27) (as a standard curve) were added to the antibody-coated wells and incubated overnight at 4°C. After washing, bound nucleosomes were detected by the addition of a monoclonal IgM reactive with H3 (28) followed by the addition of peroxidase-linked anti-murine IgM and color reagent. This assay has been shown previously to detect intact nucleosomes, but not to detect naked DNA or isolated histone components (18).

Results

Patient population

All 34 of the SLE patients were female with a mean age of 37 ± 9 yr. The RA patients were all female with a mean age of 41 ± 5 yr. We therefore enrolled only females into the control group; the mean age of this group was 35 ± 9 yr. Experiments were designed such that cells from at least one control subject were always studied at the same time as cells from RA or SLE patients, thus ensuring that our results did not occur as a result of bias or "drift" of data over time.

⁴ Abbreviations used in this paper: RA, rheumatoid arthritis; SLAM, systemic lupus activity measure; AO, acridine orange; PI, propidium iodide.

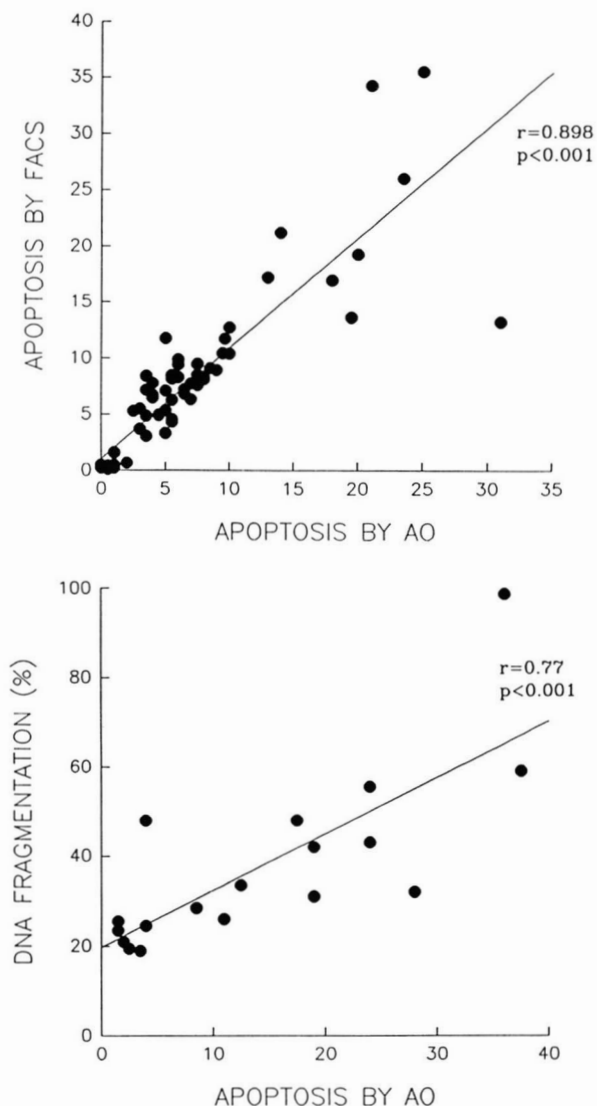


FIGURE 1. Measurement of apoptosis by different methods. (Upper panel) Relationship between apoptosis as measured by nuclear morphology after AO staining and as measured by FACS analysis after PI staining. (Lower panel) The relationship between apoptosis measured by AO staining and DNA fragmentation as measured by the diphenylamine assay. There is excellent agreement between the different methods of measuring apoptosis.

Measurement of apoptosis

Cell samples from normal controls, SLE patients, and RA patients were analyzed for apoptosis by multiple methods. The correlation between the three different methods used to measure apoptosis was excellent (Figure 1). The correlation between apoptosis as assessed by FACS analysis and AO staining showed an r value of 0.898 ($p < 0.001$) (Fig. 1a). The correlation between AO staining and DNA fragmentation measured by the diphenylamine method showed an r value of 0.77 ($p < 0.001$) (Fig. 1b). Because of this excellent agreement, results in the remainder of this

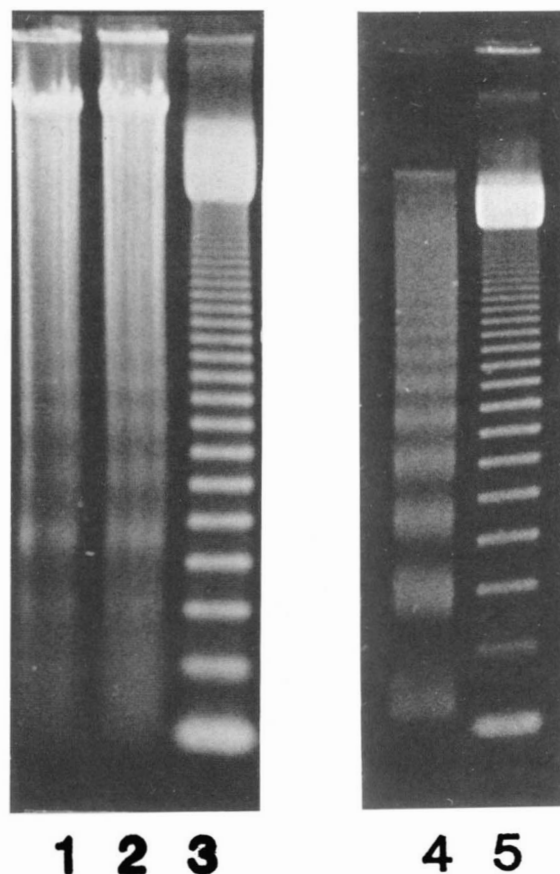


FIGURE 2. Gel electrophoresis of DNA. Lymphocytes from patients demonstrating 15 to 20% apoptosis by AO (48-h sample) were spun, and the cell-free supernatant was removed. The cell pellet was lysed, and DNA was separated by centrifugation at 13,000 rpm for 10 min (24). DNA was prepared as described in *Materials and Methods* and run on a 1% agarose gel using a 123-bp ladder as a m.w. marker (lanes 3 and 5). Lanes 1 and 2 represent cellular DNA from one SLE (lane 1) and one normal patient (lane 2). Lane 4 is DNA from the supernatant of apoptotic cells. All three samples demonstrate DNA banding at multiples of approximately 180 bp.

paper are expressed as percent apoptosis as measured by AO staining and morphologic assessment. Apoptosis was confirmed by agarose gel electrophoresis of cellular DNA showing DNA banding at multiples of 180 bp (Fig. 2, lanes 1 and 2).

Apoptosis of lymphocytes immediately after isolation

Only a small percentage of lymphocytes from each patient group showed evidence of apoptosis immediately after isolation. There was a large amount of variability in the percentage of cells that were apoptotic at t_0 within the group of SLE patients. However, the percentage of lymphocytes that were apoptotic at t_0 was significantly higher in patients with SLE than in patients with RA ($p < 0.05$) or in control subjects ($p < 0.01$ by Mann-Whitney test)

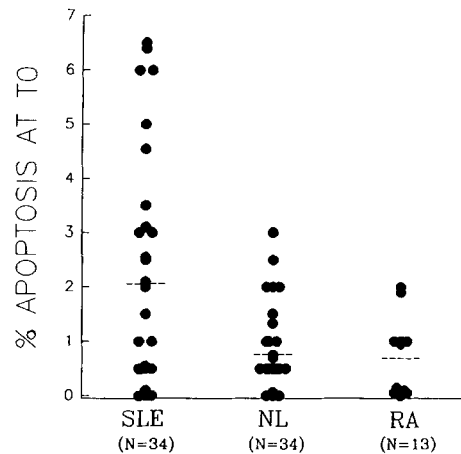


FIGURE 3. Apoptosis of lymphocytes at time 0. Immediately after lymphocyte isolation, cells were stained with AO, and the percentage of cells undergoing apoptosis was determined. Lymphocytes from patients with SLE showed a significantly higher percentage of apoptotic cells than lymphocytes from RA patients ($p < 0.05$, Mann-Whitney) or from normal controls (NL) ($p < 0.01$, Mann-Whitney).

(Fig. 3). The time required for isolation of lymphocytes was constant (4–5 h) in all patient groups, and samples from all patients were isolated and studied immediately after blood drawing. Lymphocytes isolated from patients with RA showed no increase in percent apoptosis at t_0 relative to normal controls.

Apoptosis of lymphocytes over time in culture

At each time point at which cells were removed for assessment of apoptosis, total cell counts were performed. Cell counts did not change significantly over 48 h in culture in any of the patient groups, indicating that cells undergoing apoptosis remain intact and do not significantly fragment during this time interval. Because there were no changes in total cell number, the percentage of cells that were apoptotic at any given point in time could be taken as a measure of the cumulative number of apoptotic cells in culture. Therefore, assessment of the percent of apoptotic cells at different time points allowed construction of an apoptosis-over-time curve and calculation of a rate constant based on the slope of this line.

Lymphocytes from control subjects showed a gradual increase in the percent of apoptotic cells over time in culture. At 48 h, $8.3 \pm 3.7\%$ of lymphocytes from control subjects were apoptotic. In contrast, lymphocytes from SLE patients underwent apoptosis at a much more rapid rate, such that $16.8 \pm 7.7\%$ of lymphocytes from SLE patients were apoptotic at 48 h ($p < 0.001$) (Fig. 4). Lymphocytes from patients with RA showed $8.5 \pm 4.2\%$ apoptosis at 48 h ($p < 0.01$ vs SLE).

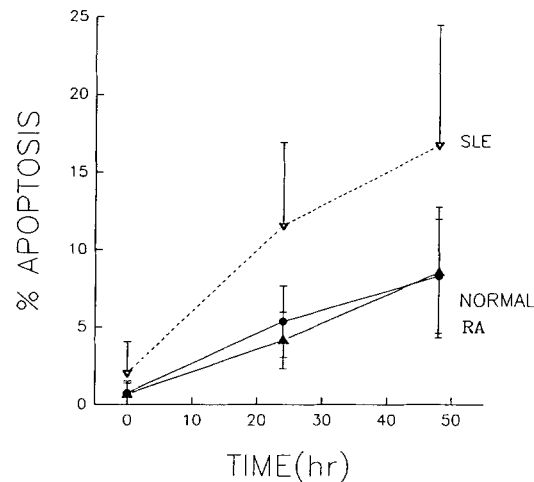


FIGURE 4. Apoptosis of lymphocytes over time in culture. Lymphocytes from patients with SLE, with RA, and normal controls were cultured at $1 \times 10^6/\text{ml}$ in RPMI/10% FCS in microtiter wells. Immediately after plating (time 0), at 24 and at 48 h, samples were removed, and the percentage of apoptotic cells was measured by AO staining. This figure shows the mean \pm SD of the percentage of apoptotic cells at each time point (normal controls, $n = 34$; SLE, $n = 34$; RA, $n = 13$). Lymphocytes from SLE patients undergo apoptosis in culture more rapidly than lymphocytes from RA patients or normal controls.

Table I. Rates of apoptosis

Group	K (slope)
Normal ($n = 34$)	0.14 ± 0.07
SLE ($n = 34$)	0.34 ± 0.18^a
Steroids ($n = 23$)	0.33 ± 0.20^b
No steroids ($n = 11$)	$0.35 \pm 0.11^{b,c}$
RA ($n = 13$)	0.15 ± 0.07^a
Steroids ($n = 9$)	0.15 ± 0.08
No steroids ($n = 4$)	0.16 ± 0.06

^a $p < 0.001$ for comparison of SLE vs normal controls or RA (t-test).
^b Difference between steroids and no steroids not statistically significant.
^c $p < 0.001$ for SLE patients on no steroids vs normal controls or RA.

The rate of lymphocyte apoptosis for each individual patient was determined by regression analysis of data at time 0, 24, and 48 h, and a slope (K , in percent of cells undergoing apoptosis per hour) was determined for each patient. The mean rate of apoptosis for lymphocytes from SLE patients was 2.4 times greater than the rate of apoptosis of lymphocytes from normal subjects ($p < 0.001$) (Mann-Whitney) and 2.3 times greater than lymphocytes from patients with RA ($p < 0.001$) (Table I). There was a considerable amount of variability in the rates of apoptosis (K value) in SLE patients, ranging from near normal to 5 to 6 times normal (Fig. 5). No correlation was found between the rates of apoptosis of lymphocytes in culture and the initial percentage of apoptotic lymphocytes at t_0 ($p = 0.361$) (data not shown).

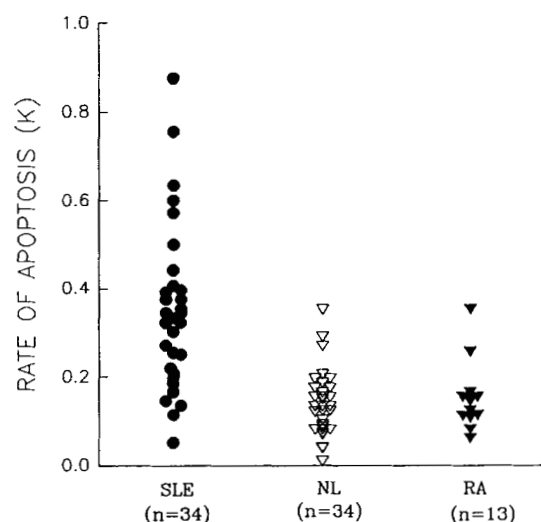


FIGURE 5. Rates of apoptosis of lymphocytes derived from individual patients with SLE, with RA, or normal controls. The rate of lymphocyte apoptosis was calculated for each patient by regression analysis of the percent of apoptotic cells at 0, 24, and 48 h. Each point on the graph represents the K value (in percent of cells undergoing apoptosis/h) for one patient.

Effect of drug therapy on apoptosis

It has been demonstrated that corticosteroids accelerate apoptosis of immature thymocytes (29) and that some cytotoxic drugs induce cell death by apoptosis (26, 30, 31). It was therefore essential to examine the effects of drug therapy on the rate of apoptosis in each of our patient groups. The percentage of lymphocytes undergoing apoptosis at t_0 did not vary in relation to steroid or cytotoxic drug administration ($p > 0.4$, data not shown). Lymphocytes obtained from SLE patients taking steroids underwent apoptosis at the same rate as lymphocytes from patients taking no steroids (Table I). Six of the SLE patients were taking > 20 mg a day of prednisone. Comparison of apoptosis of lymphocytes derived from these patients with apoptosis of cells derived from SLE patients taking no steroids showed no differences in the percent of apoptotic cells at t_0 or in rates of apoptosis. Seven of 13 patients with RA were taking steroids in doses ranging from 5 to 15 mg/day. Apoptosis of lymphocytes from RA patients taking steroids was no different from apoptosis of lymphocytes from RA patients taking no steroids (Table I). These data suggest that *in vivo* ingestion of steroids does not alter lymphocyte apoptosis and that accelerated *in vitro* apoptosis of lymphocytes derived from SLE patients occurs independent of corticosteroid therapy.

Eight SLE patients were on cytotoxic therapy in addition to corticosteroids, six on azathioprine and two on i.v. cyclophosphamide. Lymphocytes from these patients showed no increase in apoptosis at t_0 and no difference in the rate of apoptosis compared with SLE patients on no cytotoxic drugs.

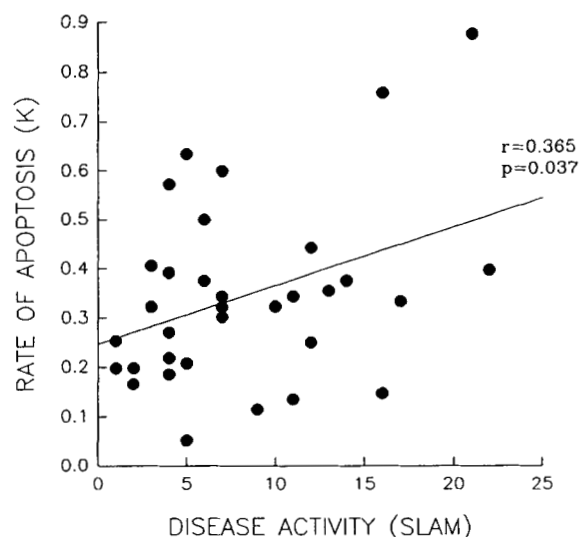


FIGURE 6. Rate of apoptosis as a function of disease activity. The rate of apoptosis (K) of lymphocytes from SLE patients was calculated by linear regression of data points at 0, 24, and 48 h. Disease activity was measured by a standardized questionnaire that included both clinical and laboratory data (SLAM). There was a significant correlation between the SLAM index and the rate of apoptosis ($p = 0.037$).

Effect of disease activity on lymphocyte apoptosis

Disease activity as measured by the SLAM was recorded at the time of blood draw for assessment of apoptosis. There was a statistically significant correlation between disease activity and the rate of lymphocyte apoptosis *in vitro* ($p = 0.037$) (Fig. 6). No correlation existed between disease activity and the degree of lymphocyte apoptosis present immediately after lymphocyte isolation ($p = 0.498$).

Release of nucleosomes

We and others have shown that nucleosomes are released into the surrounding extracellular milieu during apoptosis (10, 11, 18). The level of nucleosomes present in tissue culture supernatants at various time points was measured from all three patient groups, and the concentration of nucleosomes present in the supernatant was examined as a function of the amount of apoptosis present in culture at the time of supernatant sampling. There was a strong correlation between the amount of apoptosis as measured by AO and the nucleosome content of cell supernatants ($r = 0.729$, $p < 0.001$) (Fig. 7). Supernatants from SLE lymphocytes after 48 h in culture contained significantly greater amounts of nucleosomes than supernatants from normal lymphocytes after 48 h in culture (data not shown). Agarose gel electrophoresis of the supernatant material confirmed a nucleosomal DNA pattern with banding at multiples of 180 bp (Fig. 2, lane 4).

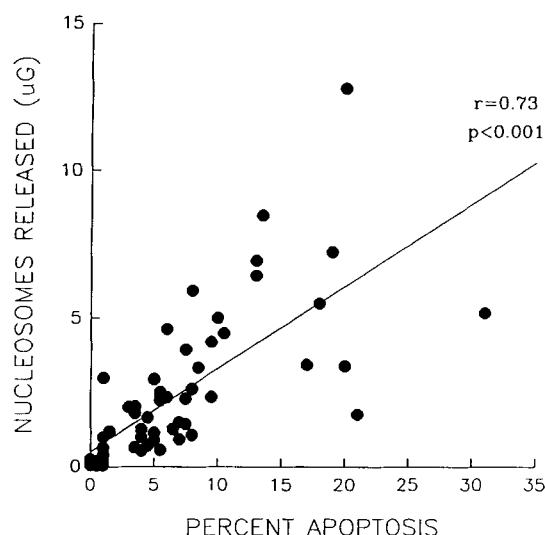


FIGURE 7. Release of nucleosomes into the culture supernatant as a function of lymphocyte apoptosis. Tissue culture supernatants were analyzed for nucleosome content by a nucleosome-specific ELISA; at the same time at which supernatants were removed for analysis, cells were assessed for apoptosis by AO staining. There is a strong correlation between the degree of lymphocyte apoptosis in culture and nucleosome release ($p < 0.001$).

Discussion

We have used three different methods to assess apoptosis of human lymphocytes over time in culture and have confirmed the presence of apoptosis in these cells by gel electrophoresis. Our data confirm the work of others that normal lymphocytes gradually undergo apoptosis when placed in tissue culture (10, 11). The major finding of this paper is that lymphocytes derived from patients with SLE undergo apoptosis in tissue culture at significantly greater rates than lymphocytes from normals. To address whether accelerated in vitro lymphocyte apoptosis is specific for SLE or is a nonspecific finding associated with chronic inflammation, we have also examined apoptosis of lymphocytes derived from patients with RA. The rate of apoptosis of lymphocytes from RA patients was indistinguishable from the rate of apoptosis of lymphocytes from control subjects, suggesting that altered lymphocyte apoptosis may be at least somewhat specific for SLE. Our data also show that altered apoptosis of lymphocytes from SLE patients did not occur as a result of drug treatment of these patients; administration of corticosteroids and cytotoxic drugs did not alter apoptosis of lymphocytes derived from either SLE patients or RA patients.

In addition to the accelerated rates of apoptosis in vitro, lymphocytes from some SLE patients also exhibited a higher percentage of apoptotic cells immediately after isolation. This result could potentially occur as a consequence of increased apoptosis of SLE lymphocytes during the isolation procedure, which required 4 to 5 h. If this were the

case, one would expect that those patients with the highest rates of apoptosis in culture would also have the highest apoptotic values at t_0 . However, there was no correlation between the rate of apoptosis in culture and the percentage of cells that were apoptotic at t_0 ($p = 0.361$), making this explanation unlikely. Increased numbers of apoptotic cells at t_0 could also potentially occur as a result of increased apoptosis of circulating cells in SLE, or because of decreased clearance of circulating apoptotic cells. Our experiments do not address these possibilities, and we are therefore unable presently to resolve this issue. The percentage of cells that were apoptotic at t_0 was not related to corticosteroid or cytotoxic drug therapy and did not correlate with disease activity as measured by the SLAM.

Our original hypothesis was that accelerated apoptosis of circulating lymphocytes in SLE patients might lead to the release of increased amounts of intact nuclear Ags, thereby providing Ag to drive an autoimmune response and to combine with Ag to form immune complexes. We have shown that intact nucleosomes are released into the extracellular milieu during apoptosis and that increased rates of apoptosis, as observed in SLE lymphocytes, are correlated with increased nucleosome release in vitro. Whether increased amounts of nucleosomes are released in vivo is still unclear, however, because one cannot assume that lymphocyte apoptosis is accelerated in vivo as well as in vitro. Our data do establish, however, that cellular apoptosis can result in the release of intact nucleosomal material into the extracellular space and thereby establishes the possibility that in vivo apoptosis may be an important source of intact nuclear Ags in diseases such as SLE. In this light, it is of interest that there was a correlation between SLE disease activity as measured by the SLAM and the rates of lymphocyte apoptosis in culture. This observation is difficult to interpret because of the arbitrary and semiquantitative nature of measures of lupus disease activity, including the SLAM. Nevertheless, our observation suggests that the more pronounced the alterations in apoptosis the more active the disease (or vice versa). One could postulate that more rapid apoptosis provides more nuclear Ag for the formation of immune complexes, resulting in increased disease activity. Such an explanation must remain purely speculative, however, until further information on in vivo apoptosis in SLE patients is forthcoming.

Accelerated apoptosis of lymphocytes from SLE patients could occur either as a result of an intrinsic abnormality of lymphocytes or as a result of an increased frequency of cells that normally undergo rapid apoptosis. B cells have been shown to undergo rapid apoptosis in vitro (33, 34), and preliminary cell separation experiments from our laboratory indicate that peripheral B cells undergo apoptosis more rapidly than T cells. Thus, if SLE patients had a higher percentage of B cells in the circulation than normals, this change in cell distribution could account for

the observed accelerated apoptosis in unfractionated lymphocytes. Data from the literature suggest, and our own data confirm that the percentage of circulating B cells is not increased in SLE patients, making this explanation unlikely (35). SLE is characterized by an increased number of activated B cells in the circulation (36, 37). Furthermore, activated cells have been shown to undergo apoptosis more readily than resting cells (38, 39), raising the possibility that rapid apoptosis of activated B cells in SLE may account for the observed accelerated apoptosis of unfractionated lymphocytes. The activation phenotype of the lymphocyte subsets undergoing apoptosis was not examined in this study, but is currently being pursued. Further experiments on the rates of apoptosis of isolated B cells, T cells, and their subsets are necessary to clearly define the basis of accelerated lymphocyte apoptosis in SLE. These studies are currently in progress.

It has recently been reported that the MRL-*lpr/lpr* murine model of SLE is characterized by an abnormality in the *fas* Ag such that signaling of apoptosis is abnormal, resulting in the failure of lymphocytes to undergo apoptosis and tissue accumulation of lymphocytes (19, 20, 21). It has been proposed that failure to peripherally delete autoreactive cells by apoptosis results in autoantibody formation and lupus-like disease (21). Correction of the *fas* defect in transgenic mice results in the restoration of normal apoptosis and the absence of autoimmunity (32). Despite the defect in *fas*-induced lymphocyte apoptosis in these mice, however, lymphocytes from *lpr/lpr* mice undergo accelerated spontaneous apoptosis in tissue culture (22). Thus, the failure to undergo apoptosis in response to one signal (*fas* ligation) does not prevent apoptosis by other mechanisms in this model. One can postulate that failure to delete cells by one mechanism (*fas*) generates a population of cells that are predisposed or primed to undergo apoptosis when the environmental conditions are altered, such as would occur with in vitro culture. The similarities between the accelerated in vitro apoptosis we have observed in lymphocytes from SLE patients and the accelerated apoptosis of MRL-*lpr/lpr* lymphocytes may merely be coincidental. Further work on the mechanisms of accelerated apoptosis in both systems is required before any parallels can be drawn. Nevertheless, one can speculate that accelerated in vitro lymphocyte apoptosis in human SLE may reflect a more fundamental in vivo defect of apoptosis, analogous to the MRL mouse model.

In MRL mice, accelerated in vitro apoptosis occurred primarily in the T cell subset, although apoptosis of B cells was not specifically examined. The data reported here demonstrate accelerated in vitro apoptosis in unfractionated lymphocytes. We are currently investigating rates of apoptosis in different lymphocyte subsets. Further work should also examine if altered apoptosis in SLE occurs solely in lymphocytes or also affects cells of other lineages such as polymorphonuclear leukocytes or keratinocytes.

Our observation of accelerated apoptosis of lymphocytes from patients with SLE provides a potential common thread between the *lpr/lpr* murine model of lupus and human lupus. Although studies of apoptosis in other human diseases are required, the apparent specificity of altered apoptosis in SLE suggests that abnormalities of apoptosis may represent a central defect in SLE. Further investigations of apoptosis in SLE should open a new chapter in lupus research and may yield important new insights into the fundamental pathogenesis of this disease.

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