Environment-Dependent Down-Modulation of CD45 Cell Surface Expression on Polymorphonuclear Cells

Ingrid Böhm*

Department of Radiology, University of Bonn, Bonn, Germany

CD45, a transmembrane protein tyrosine phosphatase, regulates important cell functions. Its involvement in clearing tissues from dead or dying cells by polymorphonuclear cells (PMNs) is unclear. Therefore, in this study the influence of exogenous factors (e.g., temperature and phagocytosis) on the CD45 expression on PMNs (in patients with lupus erythematosus (LE) vs. controls) was analysed by flow cytometry. The data showed a significant down-regulation of CD45 at 4°C in controls, but not on LE-PMNs. In the presence of targets, CD45 was significantly decreased ($P < 0.00002$). Substrate-dependent down-regulation was greater in the presence of nuclei than in the presence of dead cells

($68.98\% \pm 2.28\%$ vs. $47.89\% \pm 2.75\%$; $P < 0.002$), and on LE-PMNs than on control-PMNs ($45.93\% \pm 4.92\%$ vs. $53.65\% \pm 2.2\%$). Target pretreatment with anti-dsDNA antibody-containing serum resulted in a lower CD45 expression compared to treatment with normal serum. This effect was more pronounced on PMNs of LE patients than on those of controls. Phagocytosis was inversely correlated with CD45 expression ($P < 0.0007$). These data suggest that phagocytic clearance of cellular debris by PMNs may modulate the function of the effector cells, and that anti-dsDNA antibodies apparently enhance the phagocytic clearance. J. Clin. Lab. Anal. 18:187–194, 2004. © 2004 Wiley-Liss, Inc.

Key words: FACS-analysis; lupus erythematosus; phagocytosis

INTRODUCTION

Antigen persistence with subsequent autoantibody production has been thought to be a central event in the pathogenesis of lupus erythematosus (LE) (1). Increased amounts of circulating apoptotic cells, nucleosomes, and genomic DNA have been found in patients with LE (2–4). Consequently, the clearance of dead cells and cellular debris by phagocytosis appears to be altered in patients with LE. It has been shown that nucleosomes inhibit phagocytosis of apoptotic thymocytes by peritoneal macrophages in MRL+/+ lupus-prone mice (5). These findings highlight new aspects of biological mechanisms in cells. The authors found that apoptotic-derived particles have the ability to influence the uptake of apoptotic cells by phagocytes. The process of phagocytosis is very complex. Unfortunately, papers dealing with this subject are sparse. CD45, the common leukocyte antigen, is a cell surface glycoprotein with a transmembrane protein tyrosine phosphatase (PTPase). The CD45 molecule is known to play a variety of regulatory roles in activation-induced signalling in leukocytes. However, little is known about the behaviour of CD45 expression and function in nonlymphoid leukocytes. Therefore, in the present study, the effects of

exogenous influences on the expression of CD45 on neutrophils (PMNs) were investigated.

MATERIALS AND METHODS

Isolation of PMNs

Although many types of cells can ingest particulate material, “professional phagocytes,” such as PMNs, perform this function much more efficiently than other cells because they express unique membrane proteins and signal transduction mechanisms. In this study, human neutrophils (PMNs) were isolated from anti

Abbreviations: dsDNA, double-stranded deoxyribonucleic acid; FSC, forward scatter; LE, lupus erythematosus; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PI, propidium iodide; PMNs, polymorphonuclear cells (neutrophils); PTPase, protein tyrosine phosphatase; SSC, side scatter.

*Correspondence to: Dr. Ingrid Böhm, Department of Radiology, University of Bonn, Sigmund-Freud Strasse 25, 53105 Bonn, Germany. E-mail: i.boehm@uni-bonn.de (formerly Department of Dermatology).

Received 25 July 2003; Accepted 15 January 2004

DOI 10.1002/jcla.20021

Published online in Wiley InterScience (www.interscience.wiley.com).

coagulated whole blood provided by healthy controls and patients with LE. All patients fulfilled the American Rheumatism College criteria (ARC; formerly the American Rheumatism Association (ARA)) (6). The controls were negative for all ARC. The LE patients had a mean age of 35.5 years (range 27–48 years), and controls had a mean age of 42.4 years (range 37–55 years); this difference was not statistically significant. First, 9 mL of blood, collected in ethylenediaminetetraacetic acid tubes, were mixed with 2 mL of dextran (6% wt/vol, average molecular weight = 266,000) in phosphate-buffered saline (PBS: 10 mmol/l sodium phosphate, 2.7 mmol/l potassium chloride, and 137 mmol/l sodium chloride, pH 7.4; containing 0.4% wt/vol tri-sodium citrate). The mixture was left to stand for 60 min at room temperature to allow the red blood cells to form a sediment. This purification scheme yielded at least 98% PMNs, with few contaminating platelets and a viability of >95% as determined by trypan blue exclusion.

Autoantibody Detection

Antinuclear antibodies were detected on HEP2 cells by indirect immunofluorescence. Anti-dsDNA antibody titers were measured by direct binding to dsDNA-coated ELISA plates (VarElisaTM Pharmacia, Freiburg, Germany) according to the manufacturer's instructions, as described previously (7,8).

Target Preparation and Labelling

Density gradient centrifugation was used to separate peripheral blood mononuclear cells (PBMCs) from the whole-blood specimens. Subsequently, the cells were subjected to repeated freeze/thaw cycles to kill them. Trypan blue staining revealed that >90% of the cells died. Cell nuclei were isolated as described previously (9). Briefly, harvested PBMCs were spun at $300 \times g$ at 4°C for 5 min, and resuspended in cell lysis buffer (1% Triton X-100, 60 mM KCl, 15 mM NaCl, 14 mM β -mercaptoethanol, 300 mM sucrose, 0.5 mM EDTA, 15 mM HEPES (pH 7.5), 1 mM phenylmethylsulfonyl-fluoride in 95% ethanol, and 10 μ g/mL aprotinin) on ice for 10 min. To eliminate contamination with intact cells, the cell fraction was layered over a 400-mM sucrose gradient and spun at $1,200 \times g$ at 4°C for 10 min. The cell debris was resuspended in buffer A (200 mM sucrose, 10 mM Tris-HCl (pH 7.5), and 60 mM NaCl). Then 100 mg of PI in 1 mL of PBS were added to both the dead cells and the cellular debris. The samples were incubated at room temperature for 15 min in the dark, washed three times, and kept at 4°C in the dark until they were used. The labelling of the targets was checked

with a fluorescence microscope (Orthoplan Leitz, Wetzlar, Germany).

Flow Cytometry

Flow cytometric analysis was performed after the samples were stained with FITC-labeled anti-human CD45 mAbs (clone 2D1; Becton Dickinson, Heidelberg, Germany) and appropriate isotype controls for 20 min. After the samples were washed twice in PBS containing 0.1% bovine serum albumin and 0.01% NaN₃, they were measured on a FACScan flow cytometer (Becton Dickinson) equipped with a single 488-nm argon laser beam, with 5,000–10,000 cells acquired for each sample. Analyses and calculations were performed with the use of CellQuestTM software (Becton Dickinson). Live cells were gated on forward-scatter (FSC) vs. side-scatter (SSC) profiles. Fluorescence signals were treated by four-decade logarithmic amplification. The values were expressed as a percentage of the gated total cell population.

Phagocytosis Assay

PMNs were isolated from venous blood as described above, and the following procedure was performed (unpublished results). Briefly, RPMI 1460 culture medium was supplemented with 5% (v/v) heat-inactivated FCS, 2 mM glutamine, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 50 μ M 2-mercaptoethanol. The PMNs were cultured in a final volume of 1 mL of culture medium alone or with the above-mentioned substrate. Unless otherwise indicated, the cell cultures were performed at a cell density of 5×10^6 /well for 60 min. PI-labeled autologous targets (dead cells or cell nuclei) were added to the PMNs, and incubated at 37°C or 4°C for the indicated time. The cells were then washed with PBS to remove material that had not been engulfed. Subsequently, the cells were stained with anti-CD45 mAb FITC (clone 2D1 from BD). PI allows one to discriminate between phagocytes that have engulfed PI-stained targets, and PMNs that have not phagocytosed. Living cell membranes exclude the dye, and thus viable PMNs are not stained. Neutrophils that phagocytosed cellular targets were identified as double CD45- and PI-positive cells, whereas PMNs that did engulf the presented cellular material remained only CD45-positive. Control staining with PI after the phagocytosis revealed negative results. This was confirmed when the culture was terminated, and cell viability was assessed by trypan blue dye exclusion. The extent of phagocytosis was determined by flow cytometry. Computerized gating in FSC and SSC was used to eliminate cellular debris and dead cells from viable PMNs in the analysis. Scattergrams were generated by combining FSC with FITC green fluorescence (FL1 log). The fluorescence

histogram was divided into two regions representing the background fluorescence of nonphagocytosing cells (M1) and a high signal for cells with ingested PI-labeled targets. The cells were gated to determine phagocytosis, which was expressed as a percentage of the phagocyte-associated green fluorescent cells. The phagocytotic rates, measured as PI/CD45 double-positive cells, were graded as follows:

- 1: 0–5% engulfed PI-positive targets
- 2: 6–25% engulfed PI-positive targets
- 3: 26–50% engulfed PI-positive targets
- 4: 51–100% engulfed PI-positive targets

Statistical Analysis

Results are expressed as means $\pm$ SEM. The data were analysed by Student's *t*-test and were considered statistically significant at $P < 0.05$. To analyse the correlation between the percentage of CD45 expression and phagocytosis, a linear regression was performed and the correlation coefficient *r* was determined.

RESULTS

Temperature-Dependent Modulation of CD45 Expression on PMNs

Freshly isolated PMNs obtained from LE patients and healthy controls were washed and resuspended in PBS, and then exposed in parallel to 37°C or 4°C for 60 min. As described previously (10), exposure to 4°C induced a down-regulation of the percentage of CD45 expression on PMNs. However, while the difference between 37°C and 4°C obtained from healthy PMNs was statistically significant ($62.62\% \pm 3.58\%$ vs. $39.34\% \pm 3.48\%$; $P < 0.00002$), the decrease of CD45 observed on PMNs of LE patients was not significant ($56.56\% \pm 7.92\%$ vs. $48.01\% \pm 6.98\%$; $P = 0.426$) (Fig. 1a). Figure 1b shows the time course of the down-regulation of the CD45 expression on PMNs incubated at 4°C and 37°C. The percentage of CD45 on PMNs decreased within 60 min in parallel at 37°C and 4°C, but the reduction was more intense after the samples were incubated on ice.

Coincubation of PMNs With Substrate-Induced Down-Regulation of CD45 Expression

In the presence of targets, the percentage of CD45 on phagocytes decreased ($61.14\% \pm 3.38\%$ vs. $47.4\% \pm 3.02\%$; $P < 0.00002$) (Fig. 2a). Cocultivation with nuclei reduced the percentage of CD45 on PMNs more significantly than exposure to dead cells ($47.89\% \pm 2.75\%$ vs. $68.98\% \pm 2.28\%$; $P < 0.002$). PMNs coincubated with dead cells at 37°C vs. at 4°C produced a significant reduction of the CD45 expression ($77.0\% \pm 2.11\%$ vs. $60.97\% \pm 1.29\%$; $P < 0.000008$). A

Fig. 1. a: Percentage of CD45 expressing PMNs after a 60-min incubation at 37°C (grey bars) vs. 4°C (white bars), comparing the mean values in patients with LE vs. healthy controls. The data represent the mean CD45 expression obtained from 96 samples. The calculated differences were statistically significant ($*P < 0.00002$) in the control group, but not in the LE patients. b: Kinetic of CD45 expression on PMNs (%) at 37°C (■; upper line) and 4°C (○; lower line).

similar reduction was obtained when cellular debris was added to PMNs and then incubated at 37°C and 4°C ($58.19\% \pm 3.74\%$ vs. $37.59\% \pm 3.46\%$; $P < 0.0002$). Even the differences of the means between dead cells and cell nuclei at 37°C ($77.0\% \pm 2.11\%$ vs. $58.19\% \pm 3.74\%$; $P < 0.04$) and 4°C ($60.97\% \pm 1.29\%$ vs. $37.59\% \pm 3.46\%$; $P < 0.006$) were statistically significant (Fig. 2b).

Substrate-Mediated CD45 Expression

In the next experiment, substrate-exposed PMNs of LE patients were compared with those of healthy controls. The obtained result is presented in Fig. 3. The substrate-induced down-modulation of the CD45 expression on PMNs of LE patients was greater than that on PMNs of healthy volunteers ($45.93\% \pm 4.92\%$ vs. $53.65\% \pm 2.2\%$). A substrate-induced kinetic of the CD45 expression at 37°C showed that the maximum reduction of CD45 expression on the PMNs of a healthy control was measured after 5 min, and thereafter the percentage of CD45 expression rose again (Fig. 4a,

Fig. 2. a: Percentage of CD45 expression on PMNs is modulated in the presence of targets (with) as compared to CD45 expression before substrate exposition (without). The difference is statistically significant (* $P < 0.002$). b: CD45 expression on PMNs is differentially modulated by the type of target and the temperature used (grey bars = 37°C; white bars = 4°C). Nuclei induced a significantly lower CD45 expression (%) than the exposure to dead cells (* $P < 0.000008$; ** $P < 0.0002$; *** $P < 0.04$; **** $P < 0.006$).

upper quadrants). In contrast, the maximum reduction of CD45 expression on the PMNs of an LE patient occurred after 30 min (Fig. 4b, lower quadrants). However, due to the delayed decrease of CD45 on PMNs of the LE patient with the most pronounced reduction observed after 60 min of coincubation of PMNs with the targets, at the end of the observation period CD45 expression was lower on PMNs of the LE patient than those of the control. A substrate-induced kinetic of the CD45 expression at 4°C showed that incubation of the samples on ice resulted in the expected diminished CD45 expression on the PMNs of the healthy control and the LE patient. The more pronounced decreased on the PMNs of the control as compared to the LE patient (see also Fig. 1a) is also

Fig. 3. The decrease of CD45 on PMNs of LE patients after coincubation with substrate was more pronounced than on PMNs of healthy controls.

present in this experiment (Fig. 4b). At 4°C, the maximum decrease of the CD45 expression was measured in both the LE patient and the control after 60 min. The maximum reduction of CD45 expression on the PMNs of a healthy control was measured after 5 min, and thereafter the percentage of CD45 expression rose again (Fig. 4a, upper quadrants). In contrast, the maximum reduction of CD45 expression on the PMNs of an LE patient occurred after 30 min, and then an increase was also detectable (Fig. 4b, lower quadrants). However, due to the delayed decrease of CD45 on PMNs of the LE patient with the most pronounced decrease observed after 60 min of coincubation of PMNs and substrate, at the end of the observation period CD45 expression was lower on PMNs of the LE patient than on those of the control subject.

Serum Pretreatment of the Targets Induces Modulation of the CD45 Expression

Anti-dsDNA antibodies can affect PMNs (11). To test whether autoantibody-treated targets could modulate or influence the expression of CD45, PMNs of LE patients and healthy controls were stained for CD45 after they have engulfed different pre-treated targets. Pre-treatment of the targets has been done either with serum of healthy controls lacking both antinuclear and anti-dsDNA antibodies (designated as "normal") or with serum of LE patients containing both antinuclear (titers ranging from 1:320 to 1:2560) and anti-dsDNA antibodies (33.4 U/mL to 188 U/mL; normal range from 0 to 20 U/mL). Quite different patterns of the CD45 expression on PMNs of healthy controls were found in

Fig. 4. Kinetic of the CD45 expression (%) at (a) 37°C and (b) 4°C in the presence of nuclei (without pretreatment in serum) in a healthy control (top quadrants) and a patient with LE (bottom quadrants).

a comparison of the influences of three different targets (without serum pretreatment, with normal serum pretreatment, and with anti-dsDNA antibody-containing serum) (Fig. 5b). On these cells the CD45 expression differed only slightly, with no statistical significance

(Fig. 5b). Targets without serum pretreatment and with anti-dsDNA antibody-containing serum pretreatment induced a more pronounced decrease of CD45 on PMNs of LE patients. After pretreatment with normal serum, an increment of the CD45 expression on PMNs of LE

Fig. 5. a: Effect of serum pretreatment on the CD45 expression on human neutrophils. After the cells were killed, cellular targets were incubated for 60 min at 37°C in serum without autoantibodies obtained from healthy controls (normal) or in serum with anti-dsDNA antibodies obtained from patients with LE (anti-dsDNA). The anti-dsDNA antibody-induced down-regulation of the CD45 expression (%) on PMNs was significantly lower than after the pretreatment of the targets with normal serum (* $P < 0.002$). b: Effect of pretreatment of targets on the CD45 expression on neutrophils of LE patients (grey bars) vs. controls (white bars). CD45 expression on PMNs of controls changed only slightly when targets with a different pretreatment were used. In contrast, the CD45 expression on PMNs of LE patients was lower in the absence of serum and in the presence of anti-dsDNA antibody-containing serum than after pretreatment with normal serum (* $P < 0.004$).

patients in comparison to controls was determined (Fig. 5b).

Correlation Between Phagocytic Capacity and CD45 Expression

Phagocytosis was measured as described in Materials and Methods, with subsequent grading and correlation with the percentage of CD45 expression on PMNs. Figure 6 shows the obtained result. Phagocytosis induced a biphasic behaviour of the CD45 expression. At low amounts of ingested material (grades 1 and 2) there was an initial increase of the CD45 expression,

which induced a positive correlation coefficient $r = 0.20733$ ($P < 0.05$). After stage 2, the expression of CD45 decreased from stage 3 to stage 4 and induced a negative significant correlation ($r = -0.4605$; $P < 0.000006$) (Fig. 6).

DISCUSSION

Phagocytosis is an essential component of the final step of apoptosis and the clearance of necrotic cells. Since the disturbance of this process is thought to be crucial for the manifestation and maintaining of autoimmune diseases (such as LE) (2,12,13), the present study was undertaken to look for a cell surface molecule that shows dynamic modulation on PMNs due to phagocytosis. The cell surface expression of the CD45 molecule was chosen because of its central role in regulating many cell functions. Although the transmembrane protein tyrosine phosphatase CD45 has been intensely investigated in T and B cells, data regarding its function in neutrophils are sparse. Some studies have shown that CD45 modulates the activation of the inducible respiratory burst in normal human neutrophils (14), and is engaged in chemotaxis (15). Most excitingly, there is some evidence for the notion that CD45 may be involved in phagocytic clearance (16).

As was shown recently for the chemokine receptors CXCR1 and CXCR2 (17), phagocytosing neutrophils down-regulate the cell surface expression of CD45 (Fig. 2a). The maximum depression of CD45 was measured in parallel with the maximum phagocytosis (Fig. 6). After the substrate is engulfed, the particles must be digested inside the cell. During this phase, the PMNs appear to be refractory for the uptake of further targets (data not shown). The decrease of cell surface receptor(s) may reflect this. Although phagocytosis runs continuously, the process can be subdivided into the following phases: activation, chemotaxis, target recognition and adhesion, engulfment, fusion with phagolysosomes, digestion, and recovery or apoptosis of the phagocyte. Through regulation of chemokine receptors, CD45 can act to modulate PMN responses to chemokines. Therefore, agents that regulate CD45 can potentially modulate leukocyte traffic, and may represent a novel therapeutic approach for the treatment of inflammatory diseases (18). The influence of CD45 on chemotaxis may explain the initial up-regulation of CD45 (Fig. 6).

Adhesion of phagocytes the targets is the next step of the phagocytosis procedure. A body of evidence indicates that CD45 is involved in adhesion or recognition of the targets. Fuortes et al. (19) found adhesion-dependent protein tyrosine phosphorylation in neutrophils treated with tumor necrosis factor. In another study, adhesion of *Streptococcus gordonii* and

Fig. 6. Correlation between phagocytosis of PI-labeled targets by PMNs (for the grading scale, see Materials and Methods) and the percentage of CD45 expression on PMNs. An inverse, statistically significant ($P < 0.05$) relationship ($r = -0.20733$) was obtained.

Actinomyces naeshundii to a 200-kD PMN surface glycoprotein was identified as a leukocyte common antigen (CD45) by immunoprecipitation with a specific mAb (20). In addition to direct participation, CD45 also acts in LFA-1- and ICAM-1-mediated adhesion (21). Stibenz et al. (22) linked the CD45 molecule to the regulation of L-selectin (CD62L), a leukocyte glycoprotein important for extravasation and homotypic aggregation. While cross-linking of several adhesion molecules (CD2, CD44, $\alpha 4$ integrin, and LFA-1) by antibody did not result in a significant reduction of the expression of L-selectin, antibodies against CD45 induced loss of cell surface L-selectin within minutes, even at 4°C (23). Since the recognition of and adhesion to the targets marks an early phase of phagocytosis, activation by up-regulation of CD45 (as documented in the present study (Fig. 6)) would be expected. Engulfment of the cell surface-bound targets leads to a translocation of CD45 from the cell membrane to the cytoplasm, which is accompanied by a dramatic down-regulation of the surface expression of CD45 in the final phase of phagocytosis (Fig. 6). The dynamic translocation of the CD45 molecule was previously investigated by Pulido et al. (24). They found that polypeptides of 180 and 130–150 kDa, which were precipitated by antibodies directed against the CD45 molecule, were overexpressed on plasma membranes of neutrophils after degranulatory stimulation. This is supported by the fact that the CD45 molecule is associated with molecules of the cytoskeleton (25). Therefore, a phagocytosis-induced change of the expression of CD45 seems likely. According to previous studies (10), cells maintained at 4°C have a decreased CD45 expression (Fig. 1a). Further support for a cold-induced down-regulation is the CD45-mediated aggregation (see below) by incubation at 4°C (21). In addition to other reasons for a cold-induced down-regulation of the phagocytic capacity, the down-regulation of the CD45 molecule may

also be of some relevance. Interestingly, PMNs of LE patients as compared to controls showed a decreased reduction of the cold-induced diminished CD45 expression (Fig. 1a). These data may reflect previously reported defects of the CD45 molecule in animal models and in patients with systemic LE (SLE) (23–25). On the other hand, decreased down-regulation of the CD45 molecules may enable the PMNs of LE patients to phagocytose better at 4°C compared to controls. Interestingly, it was found that cell nuclei reduced the CD45 expression more significantly than dead cells (Fig. 2b). In parallel, an increased phagocytosis in coincubation with nuclei, as compared to dead cells, was measured. These data underscore the results of Laderach et al. (5), who showed that nucleosomes inhibit the phagocytosis of apoptotic cells. Apparently, the stimulus for the engulfment depends on the targets used. Thus, isolated nuclei appear to have a greater capacity than dead cells to stimulate phagocytes to engulf them. In other words, there apparently is a hierarchical principle behind the ingestion of phagocytotic targets. The selective uptake of targets may depend on the risk that the targets might damage the organism. This could mean that free circulating nucleosomes are more harmful than apoptotic cells. Therefore, they are preferentially eaten by phagocytes, and inhibit the uptake of apoptotic cells in a dose-dependent manner (5). Defect phagocytosis in patients with LE was reported previously (26,27). An intrinsic defect of phagocytosis could be excluded (5). However, it is evident that the altered phagocytosis depends on the severity of the LE. In the present investigation, PMNs of LE patients as compared to healthy controls showed a more pronounced reduction of the CD45 expression after coincubation with targets (Fig. 3), which runs in parallel with the phagocytic rate. Moreover, phagocytosis was greater in the patient group. Kinetic experiments showed an early nadir after 5 min in healthy controls, followed by an increase of the values. PMNs of LE patients showed a delayed reduction of the CD45 expression, with a maximal decrease at 30 min. In contrast to the cold-induced diminished down-regulation of CD45 on PMNs of LE patients, the target-induced reduction was greater in patients than in controls. Therefore, it seems likely that the mechanisms that lead to a cold- or target-induced decrease of CD45 may differ. Since anti-dsDNA antibodies play an important role in the pathogenesis of LE, their influence on the expression of the CD45 molecule was investigated in the present study. In some experiments, the targets were pretreated either with the serum of a healthy person lacking anti-dsDNA antibodies, or with anti-dsDNA antibody-containing serum. These experiments showed a more pronounced reduction of CD45

after coincubation with LE serum than with normal serum-pretreated targets (Fig. 5a). The presence of anti-dsDNA antibodies in patients with LE appeared to enhance the uptake of targets. The present results support the involvement of the CD45 transmembrane PTPase molecule in the phagocytotic process of PMNs. The ingestion of cell targets by PMNs appears to modulate the uptake process itself (28). The preferential uptake of nuclei could lead to a prolonged circulation of apoptotic or necrotic cells, which may serve as autoantibody trigger. The dynamic expression of CD45 on PMNs during phagocytosis, and the deviations of PMNs in LE patients observed in this study present new aspects for further scientific research.

REFERENCES

- Wang Y, Huang G, Wang J, Molina H, Chaplin DD, Fu YX. 2000. Antigen persistence is required for somatic mutation and affinity maturation of immunoglobulin. *Eur J Immunol* 30: 2226–2234.
- Courtney PA, Crookard AD, Williamson K, Irvine AE, Kennedy RJ, Bell AL. 1999. Increased apoptotic peripheral blood neutrophils in systemic lupus erythematosus: relations with disease activity, antibodies to double stranded DNA, and neutropenia. *Ann Rheum Dis* 58:309–314.
- Nezlin R, Dayan M, Zinger H, Mozes E. 1999. DNA levels in immune complexes circulating in mice with induced systemic lupus erythematosus. *Immunol Lett* 67:85–90.
- Williams RC, Malone CC, Meyers C, Decker P, Muller S. 2001. Detection of nucleosomes particles in serum and plasma from patients with systemic lupus erythematosus using monoclonal antibody 4H7. *J Rheumatol* 28:81–94.
- Laderach D, Bach JF, Koutouzov S. 1998. Nucleosomes inhibit phagocytosis of apoptotic thymocytes by peritoneal macrophages from MRL +/+ lupus-prone mice. *J Leukoc Biol* 64:774–780.
- Tan EM, Cohen AS, Fries JF, et al. 1982. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 25:1271–1277.
- Linnemann M, Geisen C, Menn S, Herzberg C, Dinser R, Wielckens K. 2002. Comparison of three dsDNA-ELISAs with regard to their efficiency in the diagnosis of systemic lupus erythematosus. *Clin Lab* 48:45–52.
- Pisetsky DS, Peters DV. 1981. A simple enzyme-linked immunosorbent assay for antibodies to native DNA. *J Immunol Methods* 41:187–200.
- Zhan X, Hu X, Friedman S, Maciag T. 1992. Analysis of endogenous and exogenous nuclear translocation of fibroblast growth factor-1 in NIH 3T3 cells. *Biochem Biophys Res Commun* 188:982–991.
- Chang H, Voralia M, Bali M, Sher GD, Branch DR. 2000. Irreversible loss of donor blood leukocyte activation may explain a paucity of transfusion-associated graft-vs.-host disease from stored blood. *Br J Haematol* 111:146–156.
- Hsieh SC, Sun KH, Tsai CY, et al. 2001. Monoclonal anti-double stranded DNA antibody is a leucocyte-binding protein to up-regulate interleukin-8 gene expression and elicit apoptosis of normal human polymorphonuclear neutrophils. *Rheumatology (Oxford)* 40:851–858.
- Baumann I, Kolowos W, Voll RE, et al. 2002. Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. *Arthritis Rheum* 46:191–201.
- Yu CL, Chang KL, Chiu CC, Chiang BN, Han SH, Wang SR. 1989. Defective phagocytosis, decreased tumour necrosis factor-alpha production, and lymphocyte hyporesponsiveness predispose patients with systemic lupus erythematosus to infections. *Scand J Rheumatol* 18:97–105.
- Liles WC, Ledbetter JA, Waltersdorff AW, Klebanoff SJ. 1995. Cross-linking of CD45 enhances activation of the respiratory burst in response to specific stimuli in human phagocytes. *J Immunol* 155:2175–2184.
- Harvath L, Balke JA, Christiansen NP, Russell AA, Skubitz KM. 1991. Selected antibodies to leukocyte common antigen (CD45) inhibit human neutrophil chemotaxis. *J Immunol* 146:949–957.
- Yu C, Yu HS, Sun KH, Hsieh SC, Tsai CY. 2002. Anti-CD45 isoform antibodies enhance phagocytosis and gene expression of IL-8 and TNF-alpha in human neutrophils by differential suppression on protein tyrosine phosphorylation and p56lck tyrosine kinase. *Clin Exp Immunol* 129:78–85.
- Doroshenko T, Chaly Y, Savitskiy V, et al. 2002. Phagocytosing neutrophils down-regulate the expression of chemokine receptors CXCR1 and CXCR2. *Blood* 100:2668–2671.
- Mitchell GB, Khandaker MH, Rahimpour R, et al. 1999. CD45 modulation of CXCR1 and CXCR2 in human polymorphonuclear leukocytes. *Eur J Immunol* 29:1467–1476.
- Fuortes M, Jin WW, Nathan C. 1993. Adhesion-dependent protein tyrosine phosphorylation in neutrophils treated with tumor necrosis factor. *J Cell Biol* 120:777–784.
- Ruhl S, Cisar JO, Sandberg AL. 2000. Identification of polymorphonuclear leukocyte and HL-60 cell receptors for adhesions to *Streptococcus gordonii* and *Actinomyces naeslundii*. *Infect Immun* 68:6346–6354.
- Lorenz HM, Harrer T, Lagoo AS, Baur A, Eger G, Kalden JR. 1993. CD45 mAb induces cell adhesion in peripheral blood mononuclear cells via lymphocyte function-associated antigen-1 (LFA-1) and intercellular cell adhesion molecule 1 (ICAM-1). *Cell Immunol* 147:110–128.
- Stibenz D, Bührer C, Laufer D, Obladen M. 1996. CD45 engagement induces L-selectin down-regulation. *Scand J Immunol* 44:37–44.
- Wroblewski M, Hamann A. 1997. CD45-mediated signals can trigger shedding of lymphocyte L-selectin. *Int Immunol* 9:555–562.
- Pulido R, Lacal P, Mollinedo F, Sanchez-Madrid F. 1989. Biochemical and antigenic characterization of CD45 polypeptides expressed on plasma membrane and internal granules of human neutrophils. *FEBS Lett* 249:337–342.
- Shao JY, Hochmuth RM. 1999. Mechanical anchoring strength of L-selectin, beta2 integrins, and CD45 to neutrophil cytoskeleton and membrane. *Biophys J* 77:587–596.
- Pablos JL, Gomez-Reino JJ. 1998. Defective phagocytosis in SLE. *Eur J Clin Invest* 28:864–865.
- Hermann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR. 1998. Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. *Arthritis Rheum* 41: 1241–1250.
- Erwig LP, Gordon S, Walsh GM, Rees AJ. 1999. Previous uptake of apoptotic neutrophils or ligation of integrin receptors downmodulates the ability of macrophages to ingest apoptotic neutrophils. *Blood* 93:1406–1412.