Figure S1.  Specificity of PCNA detection by Western blot analysis in neutrophils and PCNA expression in monocytes and lymphocytes.
(A) Neutrophils (PMN) and HeLa cells were analyzed by Western blotting with either the rabbit anti-PCNA pAb (Ab5) or the mouse mAb (PC10) as primary antibodies at 1 μg/ml concentration. PCNA appears as a single 36-kD band in both cell types (left). When both anti-PCNA (Ab5 or PC10) had been preincubated for 3 h with 10 μg/ml purified recombinant PCNA to preclude further PCNA detection (right), no PCNA could be detected by Western blot analysis. Notably, to obtain signals of equal intensity, the amount of loaded protein was 50 μg and 10 μg for PMN and HeLa, respectively. This experiment has been performed three times with the same results. (B) Confocal microscopy immunofluorescence detection of PCNA, using the pAb Ab5 in neutrophils and HeLa cells. Nuclei were visualized with TO-PRO 3 iodide. Data show representative images of at least 10 experiments yielding the same results. (C) Western blot analysis of PCNA in cytosolic (Cyt) and nuclear (Nu) fractions prepared from lymphocytes and monocytes. Lamin B and tubulin were used as controls for the nuclear and the cytosolic fractions, respectively. (D) Immunofluorescence detection by confocal microscopy of PCNA performed as in B in lymphocytes and monocytes. Bars, 10 μm.
Figure S2. PCNA subcellular localization during ATRA-induced granulocyte differentiation in NB4 cells. (A) Confocal microscopy immunofluorescence detection of PCNA using the Ab5 pAb as in Fig. 1 C during ATRA-induced granulocytic differentiation of promyelocytic NB4 cells. Bars, 10 μm. (B) Bar graphs show percentages of cells exhibiting nuclear, mixed nuclear–cytoplasmic, or cytoplasmic PCNA localization, as determined by counting the cells (n = 300) under the microscope (**, P < 0.01, Student t test). Error bars represent SEM. A and B show a representative experiment out of three yielding the same results.
Figure S3. Neutrophil apoptosis assessment by phosphatidylserine externalization or mitochondrial depolarization. Neutrophil apoptosis occurred after a 15-h incubation at 37°C (constitutive apoptosis) or was potentiated by anti-Fas mAb or gliotoxin. (A) Phosphatidylserine externalization measured by flow cytometry after annexin-V and 7-AAD labeling. Neutrophils gated into the bottom right quadrant of each panel were annexin-V⁺ and 7-AAD⁻ and thus considered apoptotic and not necrotic. (B) Assessment of mitochondrial depolarization after DiOC₆ labeling under basal conditions taken as negative control (thin lines) and after physiological (constitutive) or anti-Fas- or gliotoxin-triggered apoptosis (bold lines). The percentages of cells with decreased DiOC₆ labeling showing depolarized mitochondria were measured. A and B show one representative experiment among >20 performed yielding the same results.
Figure S4. Implication of proteasome or cathepsin inhibitors on PCNA expression. (A) PCNA expression by Western blot analysis in neutrophils, either freshly isolated (fresh) or incubated for 15 h at 37°C (apoptosis) to induce constitutive apoptosis, without (0) or with 100 μM MG132. On the top, a representative PCNA immunoblot is shown. On the bottom, neutrophil survival was evaluated as the percentage of annexin-V− 7-AAD− neutrophils, to exclude cell apoptosis and necrosis, and compared with freshly isolated neutrophils. Values are means ± SEM of five independent experiments performed in duplicate (*, P < 0.05; Student’s t test). (B) PCNA Western blot analysis after IP of neutrophil cytosol under basal conditions or after 1-h incubation at 37°C using an antiubiquitin mouse mAb (IP ubiquitin) or a control IgG (IP control) antibody. Unbound (UB) and bound (B) materials were analyzed by Western blot using the rabbit Ab5 pAb. (C) Effect of cathepsin inhibition by chymostatin on PCNA expression at the indicated concentrations as in A. Error bars represent SEM.
Figure S5. PCNA expression in mouse neutrophils undergoing apoptosis. (A) Confocal microscopy immunofluorescence detection of PCNA using the Ab5 in mouse neutrophils isolated from BM or from peripheral blood. Bar, 5 μm. (B) Analysis of PCNA protein expression in BM-isolated mouse neutrophils treated with increasing gliotoxin concentrations. Western blot analysis of procaspase-3 was performed using neutrophil cytosolic fractions, and colloidal gold staining of the membrane was used as loading control (LC). The percentages of viable neutrophils (which were not labeled by FITC-conjugated annexin-V) are shown on the top. This representative experiment has been performed three times with similar results.

Figure S6. Decreased cytoplasmic PCNA expression in neutrophils undergoing carboxyp21 peptide–induced apoptosis. Neutrophils were treated with or without (basal) the carboxyp21 peptide at 50 μM for 3 h as described in Fig. 5 E. Cell morphology and integrity are displayed in the left panels by phase-contrast microscopy, whereas apoptosis-induced nuclear morphological changes are displayed in the middle panels by Hoechst staining. Cytoplasmic PCNA expression is instead displayed by confocal microscopy immunofluorescence using the Ab5 antibody. A representative experiment out of three performed with similar results is shown. Bars, 5 μm.
Figure S7. Phenotypic and functional analysis of neutrophil-differentiated PLB985 cells stably overexpressing PCNA. PLB985 cells were treated with DMF for 5 d to induce granulocyte differentiation. (A) Confocal microscopy analysis of immunofluorescence PCNA detection using the Ab5 pAb in PLB985 cells before and after DMF treatment. The panel shows one representative experiment out of 10 performed with the same results. Bars, 5 μm. (B) CD11b expression measured by flow cytometry and expressed as the percentage of CD11b+ cells. (C) NADPH-oxidase activity. PMA-triggered superoxide anion production was assessed by lucigenin-amplified chemiluminescence in control and PCNA-transfected PLB985, before (−DMF) and after DMF-induced differentiation (+DMF). Results are reported as the ratio of lucigenin-amplified chemiluminescence obtained without over that obtained with PMA. (B and C) Data are mean ± SEM of five independent experiments.
Figure S8. Association and colocalization of PCNA with procaspase-3, procaspase-8, procaspase-9, and procaspase-10. (A–D) Reverse co-IP experiments were performed as described in Fig. 8 except that here procaspase-3 (A), procaspase-8 (B), procaspase-9 (C), and procaspase-10 (D) were immunoprecipitated using specific mouse mAbs, whereas PCNA Western blot analysis was performed in the unbound (UB) and bound (B) immunoprecipitated proteins using the rabbit Ab5 pAb. Procaspases were also detected by Western blot analysis as a positive control. Colocalization of PCNA and procaspases in neutrophil cytoplasm are shown on the right panels. Confocal microscopy analysis of indirect fluorescence immunolabeling of PCNA (red) and procaspase-3, procaspase-8, procaspase-9 and procaspase-10 (green) was performed on freshly isolated neutrophils. Nuclei were labeled with Hoechst (blue). The overlay fluorescence (yellow) showed that PCNA colocalizes with each caspase within the cytosol. A–D show representative experiments that have been performed at least three times with similar results. Bars, 5 μm.