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In vitro inhibition of natural-killer-mediated lysis by chromatin fragments

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Abstract. A qualitative impairment of natural killer (NK) function and the presence of circulating DNA have been independently reported in clinical situations such as cancer and lupus. The existence of receptors for chromatin fragments at the leukocyte membrane raised the question of the relation between the presence of chromatin fragments in the extracellular medium and the impairment of NK function. The present study shows that plasmas from patients with metastatic cancer and with pathological DNA concentrations inhibited significantly the NK activity of normal lymphocytes as compared to cancer plasmas with DNA concentrations in the normal range. In vitro, it was demonstrated that chromatin fragments inhibited the NK-mediated cytotoxicity in a dose-dependent manner. Inhibitory concentrations of nucleosomes (2.5–10 μg/ml) were lower than those of DNA and histones alone (100 μg/ml). Inhibitory effects of nucleosomes, DNA and histones differed also according to the effector population used: nucleosomes were effective whatever the CD56+ cell enrichment of the effector population, while DNA inhibition needed T cells, and histone inhibition probably resulted from a subtoxic effect, prevented by the presence of adherent cells. Finally we found that nucleosomes could inhibit the NK function only when they were present in the extracellular medium. Taken together, these data suggest that the persistence of nucleosomal DNA at sites of cell death or in the blood might be responsible, at least partly, for the NK activity impairment observed in pathological circumstances characterized by a high rate of cell death phenomena such as cancer.

Key words: Natural killer cell – Nucleosome – DNA – Histones – Cell death

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Introduction

Natural killer (NK) cells are CD3⁻ CD16⁺ CD56⁺ large granular lymphocytes, that spontaneously kill tumour or virus-infected cells without prior sensitization and without restriction by major histocompatibility antigens.

An inhibition of NK function has been reported in many clinical situations such as cancer [5, 15, 17, 20, 28], AIDS [26], systemic lupus erythematosus [4, 10, 32] or even surgical resection [15, 21, 22]. In most cases, a qualitative impairment rather than a decreased number of NK cells has been reported. This impairment could result from various NK-suppressive factors including cytokines [19, 32], blocker antibodies [18, 20] and others factors that have not yet been determined [5, 13, 15].

A common denominator of all these clinical situations is the presence of a high cell death level, responsible for the release into extracellular spaces of cell death products that could go beyond the physiological mechanisms of clearance. Hence, we and others have found increased concentrations of plasma DNA in patients suffering from cancer with metastasis [9], systemic lupus erythematosus [8, 24], surgical resection (reviewed in [8]) and AIDS (unpublished results from our laboratory). Moreover, chromatin fragments can specifically bind to the leukocyte surface [2, 11, 14, 23] and could alter their functions.

In this study, we address the question of the possible effects of chromatin fragments on NK cell function.

Materials and methods

Reagents. Histones isolated from calf thymus (equimolecular quantities of the five histones) and calf thymus DNA were purchased from Boehringer-Mannheim (Germany).

Plasma samples. DNA concentration was determined in plasmas collected with informed consent from ten patients suffering from metastatic cancer (stage IV, seven lung carcinomas, one kidney adenocarcinoma, one rectal carcinoma and one Hodgkin's disease) and free of any feature known to be associated with increased plasma DNA concentration. Plasma samples from five healthy donors were used as

controls. The presence of alloantibodies and autoantibodies against MHC class I antigens was tested using a panel of 24 different phenotypes in a standard microcytotoxicity method as described [29].

DNA assay. DNA was assayed using a new enzymatic method [6] validated by reference to [7]. Briefly, DNA from plasma samples was adsorbed onto a histone-H1-coated microtitre plate and biotinylated in a nick-translation reaction. DNA was then quantified photometrically at 450 nm using streptavidin-conjugated peroxidase and tetramethylbenzidine. Using this method the upper limit of physiological plasma DNA concentration was 30 ng/ml.

Nucleosome assay. A nucleosome assay was also developed to ascertain that, in the plasma studied, extracellular DNA circulated in the form of nucleohistone complexes. In this assay, microtitre plates were sensitized with a monoclonal anti-histone antibody (clone H11-4 from Boehringer-Mannheim), allowing the absorption of nucleosomes through their histone component. After washing, the DNA component was biotinylated and quantified as described in the DNA assay. Quantification of nucleosomic DNA was performed by reference to standards made from a pool of plasmas containing 5 µg/ml nucleosomic DNA collected from lipopolysaccharide-injected OF1 mice (Iffa Credo, Saint-Germain-sur l'Arbresle, France) as described [7]. The sensitivity of the nucleosome assay was lower than that of the DNA assay. It allowed the detection of nucleosomic DNA above the normal range (>30 ng/ml) while the DNA assay allowed quantification in the normal range (5–30 ng/ml).

Nucleosome preparation. Mononucleosomes were obtained from chinese hamster ovary (CHO) cells. The cell pellet (4×10^8 CHO cells) was suspended in 30 ml buffer A (250 mM sucrose, 10 mM TRIS/HCl pH 7.4, 2.5 mM $MgCl_2$, 0.1 mM $CaCl_2$) and lysed at 4° C an Ultraturraxhomogenizer, 0.3% Nonidet P40 and 4 mg collagenase. After washing in buffer A (centrifugation at 800 *g*, 4° C, 5 min), the pellet was resuspended and washed in buffer B (340 mM sucrose, 15 mM TRIS/HCl pH 7.4, 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 2 mM $CaCl_2$) then resuspended in 4 ml buffer B and digested by S7 nuclease (Boehringer, 200 U/ml, 5 min, 37° C) in the presence of $CaCl_2$ (final concentration 2 mM). The reaction was stopped by EDTA (final concentration 10 mM). After centrifugation (800 *g*, 4° C, 5 min), the supernatant was harvested and mononucleosomes were separated by ultracentrifugation (SW28 rotor, Beckman, 27000 rpm, 4° C, 16 h) using an 8%–35% sucrose gradient in buffer C (50 mM TRIS/HCl, 1 mM EDTA, 1 mM dithiothreitol, 0.6 mM leupeptin pH 8). Collected fractions were dialysed in phosphate-buffered saline (PBS), DNA size and the presence of histones were checked by electrophoresis. Nucleosome concentration was established using spectrophotometry at 260 nm.

Effector cells. Peripheral blood mononuclear cells (PBMC) were isolated as previously described [3]. Briefly, blood samples were layered on a Ficoll/Hypaque density gradient (Pharmacia, France) and PBMC were resuspended after washing in culture medium: RPMI-1640 medium (Gibco, Grand Island, N.Y.) supplemented with heat-inactivated fetal calf serum (10% FCS, Gibco), 2 mM L-glutamine (Gibco), and 50 U/ml penicillin/50 mg/ml streptomycin (Biomerieux, France). Peripheral blood lymphocytes (PBL) were obtained by removing adherent cells from PBMC through adhesion on plastic culture dishes (3×10^6 cells/ml in culture medium, 2 h, 37° C, 5% CO_2 atmosphere). A NK-enriched population was obtained by CD3 cell depletion using sheep anti-(mouse IgG) Dynabeads (Immunotech, France), precoated with anti-CD3 monoclonal antibody (mAb) from Immunotech (5 µg mAb/mg beads in PBS/0.2% bovine serum albumin, 15 min, 20° C). After washing, PBL (10^7/ml in PBS/30% FCS) were incubated for 5 min with Dynabeads (1 mg beads/10^7 cells), then placed on a magnet for 10 min, unrosetted cells ($CD3^+$-population) were collected.

Quantification of $CD3^-$ cells after magnetic depletion from a PBL suspension. NK enrichement was quantified by flow cytometry. Staining was performed in PBS with anti-CD3 or anti-CD56 mAb (Immunotech) (2.5 µg mAbs/5×10^5 cells in 200 µl for 20 min at 4° C). After washing, cells were incubated for 20 min with fluorescein-labelled anti-(mouse Ig) $F(ab')_2$ (Immunotech), washed and fixed in PBS/1% paraformaldehyde before analysis using a FACScan cell sorter (Becton-Dickinson, Mountain View, Calif.), equipped with an argon ion laser operating at an emission wavelength of 488 nm. Gates were established to exclude dead cells and cell debris from analysis. Samples containing 10^4 cells from each sample were analyzed using autofluorescence of cells only incubated with fluorescein-$F(ab')_2$ as the negative control. Data were expressed as the percentage of positive fluorescent cells.

Table 1. Effect of plasmas from patients with cancer on natural-killer(NK)-mediated cytotoxicity

Patients	DNA (ng/ml)	Cytotoxicity (%)
Healthy (n = 5)	18±2	21±6
Cancer (n = 5)	20±4	20±5
Cancer (n = 5)	156±53*	6±1*

PBMC from healthy donors were used as effector cells (E/T ratio = 200) in a ^{51}Cr-release assay performed in plasmas from healthy donors and metastic cancer patients. The DNA concentration was determined in the different plasmas. Percentage cytotoxicities observed in the plasmas were sorted according to the limit of pathological DNA concentration (>30 ng/ml)

* The results are means ± SEM from two independent experiments.

* P <0.05 as compared to healthy donors

Chromium-release assay (^{51}Cr test). Erythroleukaemic K562 cells were used as target cells: 2×10^6 cells were labelled (1 h at 37° C) using 100 µCi $Na^{51}CrO_4$ (Amersham, France). After washing, target cells were adjusted to 10^5/ml in culture medium and added to U-bottomed wells in a 96-well microplate (Falcon, Gibco) at 100 µl/well. Effector cells, from 5×10^6 to 5×10^5 cells/ml in culture medium, were added at 100 µl/well, to obtain different effector : target cell ratios (from 50 : 1 to 5 : 1). In some experiments culture medium was substituted by plasmas (from cancer patients and healthy donors). After a 4-h incubation (37° C, 5%, CO_2 atmosphere) microplates were centrifuged (1500 rpm, 5 min, 4° C) and 100 µl supernatant was collected to quantify radioactivity using a gamma scintillation counter (Packard). Spontaneous and maximal chromium release were evaluated using target cells only. Percentage cytotoxicities were calculated as follows: (experimental release – spontaneous release) ×100/(maximal release – spontaneous release). Cytotoxic potentials of effector cells, calculated from individual E:T curves, were expressed as $LU_{20}/10^6$ effector cells. One LU_{20} is defined as the number of effector cells required to lyse 20% of 10^4 target cells.

Statistical analysis. Experiments were performed in triplicate. Results were expressed as means ± SEM. Statistical analysis was established using Student's paired t-test.

Results

Inhibition of NK-mediated lysis by plasmas from patients with metastatic cancer

DNA assays and ^{51}Cr cytotoxicity tests were simultaneously performed using plasma samples from cancer patients. The NK activity evaluated in plasmas was sorted according to the DNA concentration. As shown in Table 1, a significant inhibition of the NK activity was observed in cancer patient plasmas with pathological DNA concentra-

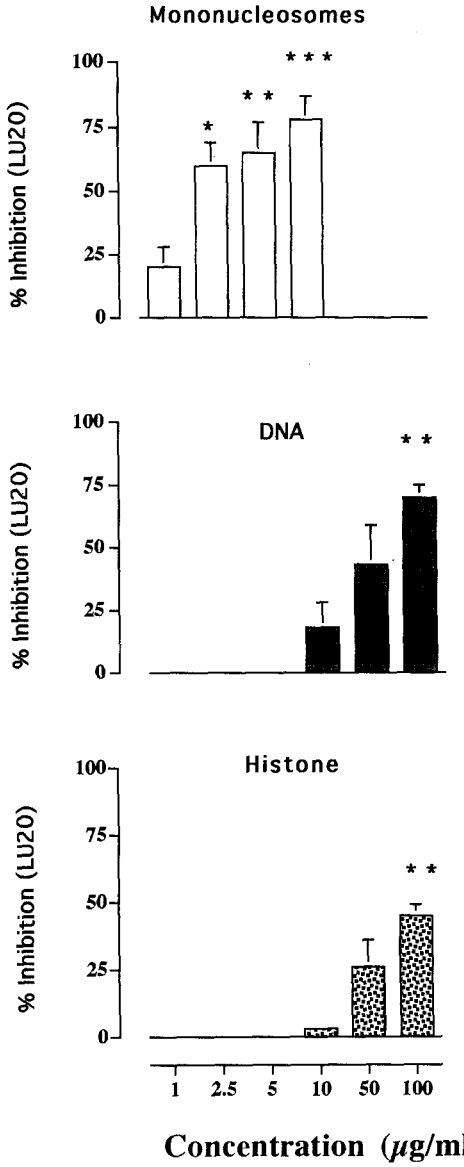

Fig. 1. Dose-dependent inhibition of natural-killer(NK)-mediated lysis by chromatin fragments. NK cytotoxicity of a peripheral blood lymphocyte (PBL) suspension was assessed in 4-h ^{51}Cr tests using K562 cells as target cells in the presence of chromatin fragments added to the culture medium: □, Mononucleosomes; ▨, DNA; ▩, histones. Nucleosomes were tested up to 10 μg/ml; DNA and histones were not inhibitory below 10 μg/ml. Results are expressed as percentage inhibition (mean ± SEM) relative to controls without chromatin fragments and calculated from cytotoxic potentials (LU_{20}; four independent experiments). Statistical analysis was carried out using Student's paired *t*-test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ versus control

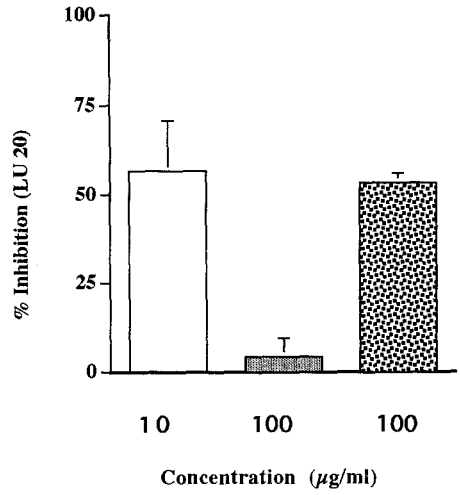

Fig. 2. Inhibition of NK-mediated lysis by chromatin fragments in NK-enriched PBL. NK cytotoxicity was assessed in a 4-h ^{51}Cr test, in the presence of chromatin fragments added to the culture medium: □, Mononucleosomes 10 μg/ml; ▨, DNA 100 μg/ml; ▩, histones 100 μg/ml. Results are expressed as percentage inhibition (mean ± SEM) relative to controls without chromatin fragments and calculated from cytotoxic potentials (LU_{20}; two independent experiments)

Table 2. DNA quantification in six plasma samples from cancer patients using the DNA and the nucleosomic assays

Patients	Plasma DNA concentration (ng/ml)	
	DNA assay	Nucleosomic assay
1	10	<30
2	25	<30
3	180	100
4	25	<30
5	220	150
6	105	140

DNA and nucleosomic assays were performed as described in Materials and methods. DNA concentrations were determined by reference to standard curves constructed from pBR 322 DNA (DNA assay) and from plasma from lipopolysaccaride-injected OF1 mice with a known amount of circulating nucleosomic DNA (nucleosomic assay)

tions (156±53 ng/ml: $P < 0.05$ as compared to plasma from healthy volunteers and to plasma from cancer patients with a plasmatic DNA concentration in the normal range). Six plasmas from previous patients suffering from cancer were also studied in the nucleosomic assay. Results shown in Table 2 indicate that, as in other pathological situations in which plasma DNA has been characterized [8, 24], in cancer patients extracellular DNA circulates in the form of nucleohistone complexes. Moreover, antibodies to HLA class I antigens were detected in patients with high plasma DNA concentrations (2/5) as well as in patients with plasma DNA concentrations in the normal range (2/5), indicating that their presence cannot explain the inhibitory effect of plasmas with pathological DNA concentrations.

In vitro inhibition of NK-mediated lysis by chromatin fragments

The cytotoxic activity of PBL suspensions was assayed in 4-h ^{51}Cr tests. As shown in Fig. 1, chromatin fragments decreased the NK potential in a dose-dependent manner. The NK cytotoxicity inhibition induced by nucleosomes appeared significant at 2.5 μg/ml while DNA and histones inhibited the NK function only at concentrations of 100 μg/ml. In the presence of 10 μg/ml nucleosomes, the cytotoxic potential, expressed as lytic units (LU_{20}), was found to

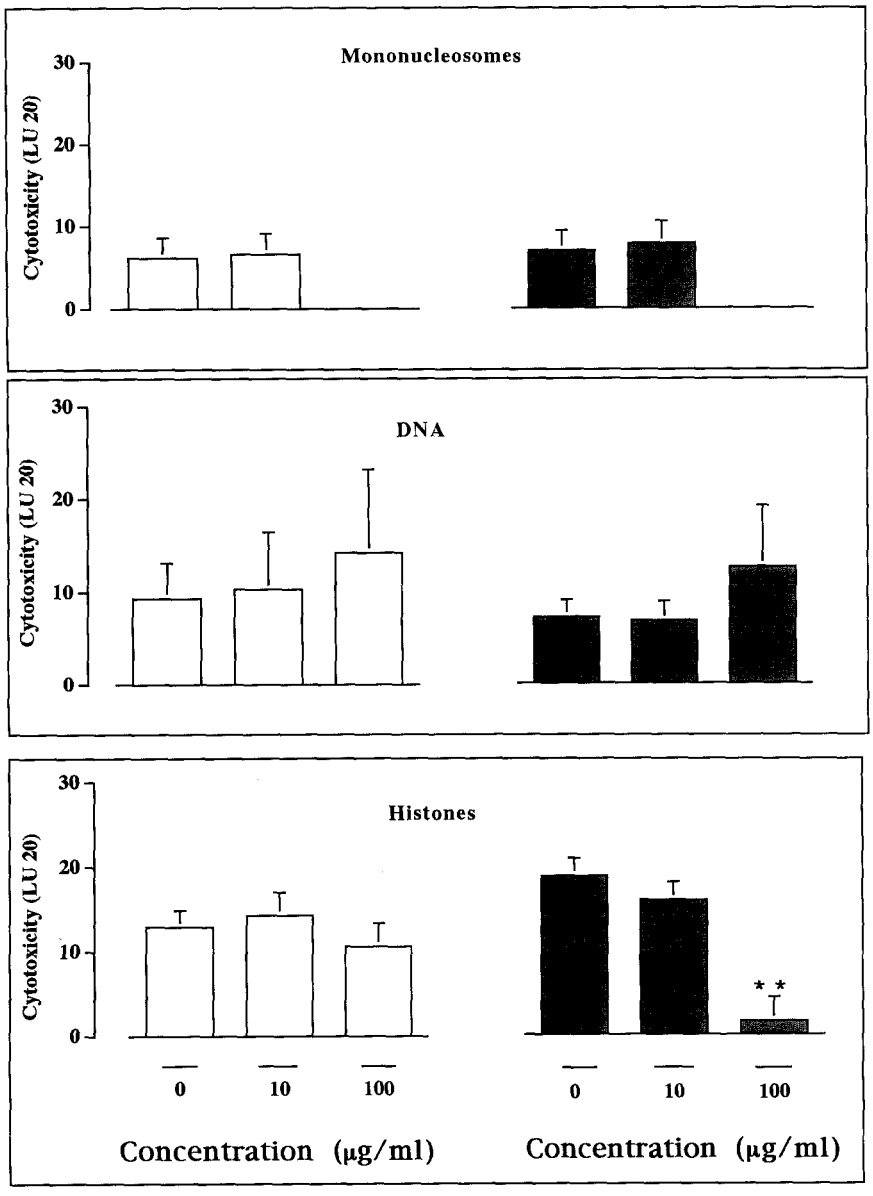

Fig. 3. Effect of a 24-h effector cell preincubation with chromatin fragments. Effector cells, peripheral blood mononuclear cells (PBMC, □) or PBL (■) were preincubated for 24 h with chromatin fragments (mononucleosomes, DNA, histones) added to the culture medium at different concentrations (10 µg/ml: mononucleosomes, DNA and histones; 100 µg/ml: DNA and histones) and washed before the ^{51}Cr test. The cytotoxic potential of effectors cells was expressed as LU_{20}; mean ± SEM of at least four independent experiments. Statistical analysis was carried out using Student's paired *t*-test: $^{**}P < 0.01$

decrease from 13.9±3.0 to 3.0±0.4 LU_{20} ($P < 0.001$) whereas 100 µg/ml DNA reduced the cytotoxic potential from 13.9±3.0 to 4.3±0.4 LU_{20} ($P < 0.01$). Histones at 100 µg/ml decreased the cytotoxic potential from 8.8±0.6 LU_{20} in controls to 4.9±0.9 LU_{20} ($P < 0.01$).

To determine whether chromatin fragments were effective on natural killer cells, ^{51}Cr tests were performed using $CD3^-$ PBL as effector cells ($CD56^+$ above 70%). In these conditions, only mononucleosomes and histones strongly inhibited the NK function. In contrast, no inhibitory effect was detected with DNA (Fig. 2).

Effects of chromatin fragments on effector cells

Nucleosomes, histones and DNA clearly inhibited the NK function in a PBL population when added in the presence of both effector and target cells. To determine whether this inhibition resulted from an interaction of chromatin fragments with effector rather than with target cells, PBMC and PBL were incubated for 24 h with the different chromatin fragments. After incubation and washing, NK function was unaltered in PBL and PBMC incubated with DNA or nucleosomes. In contrast, NK activity was significantly ($P < 0.01$ versus controls) decreased in PBL incubated with histones at 100 µg/ml (Fig. 3). Taken together, these results indicated that nucleosomes and DNA induced a reversible modification of NK function, whereas incubation with histones decreased durably the NK-mediated cytotoxicity.

Similarly, after a 24-h incubation with chromatin fragments, the assessment of PBL viability by trypan blue exclusion showed that DNA (up to 100 µg/ml) and nucleosomes (at 10 µg/ml) did not alter cell viability (more than 98% cell viability), whereas histones seemed toxic (80% cell viability in some experiments). Though the inhibitory effect of histones did not significantly correlate with the decrease in viability in individual cell suspensions (not shown), NK-activity impairment induced by histones could

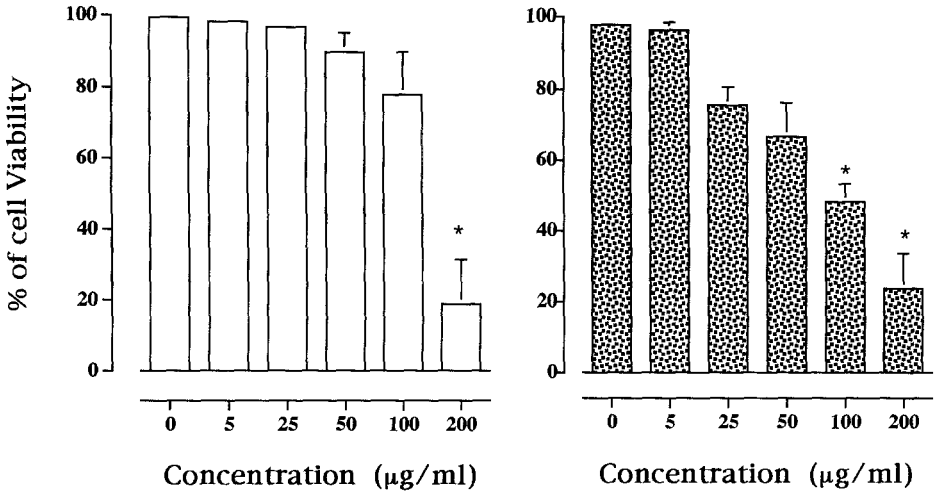

Fig. 4. Assessment of PBL viability by trypan blue exclusion after incubation with histones and poly-L-lysine. PBL were incubated for 24 h with (□) histones and (▨) poly-L-lysine. Percentage viability is expressed as the mean ± SEM of four independent experiments. *$P < 0.05$ versus control PBL

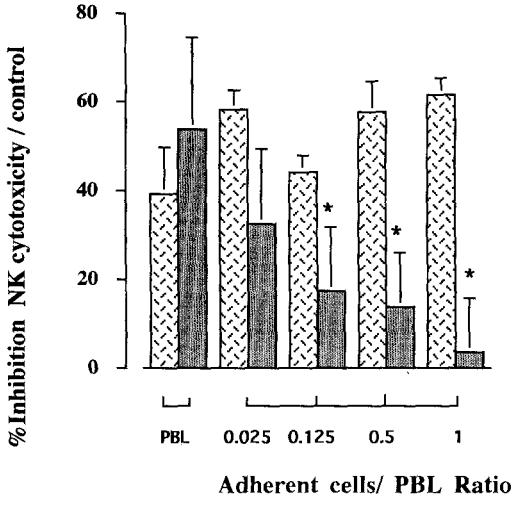

Fig. 5. Role of adherent cells on the inhibition of NK activity induced by chromatin fragments. NK cytotoxicity was assessed in a 4-h ^{51}Cr test at an effector:target ratio of 25, in the presence of (▨) 100 µg/ml DNA or (▨) 100 µg/ml histones. Effector cells consisted of a fixed number of PBL with an increasing number of adherent cells, isolated from the same PBMC suspension. Results (percentage inhibition relative to the cytotoxicity in controls without chromatin fragments), were expressed as the mean ± SEM of five independent experiments. *$P < 0.05$ versus PBL with chromatin fragments

result from a subtoxic effect on effector cells, mostly still alive (by trypan blue exclusion) but unable to kill target cells. The different effects of different chromatin fragments on cell viability could result from a difference in ionic interactions with the electronegative cell membrane [25]. Indeed, at physiological pH, DNA is anionic, nucleosomes are neutral while histones are cationic. To explore such a possibility, the effects of histones and poly-L-lysine (a polycation of 15–30 kDa) were compared. In both cases, the cell viability was decreased in a concentration-dependent manner (Fig. 4) with significant cell death ($P < 0.05$ versus controls) at 100 µg/ml for poly L-lysine and 200 µg/ml for histones. This comparative study suggested that the toxic effect induced by histones was due to an excess of positive charges from basic peptides, possibly interfering with the cell membrane negative charges.

Role of adherent cells on the inhibitory effect of chromatin fragments

The inhibition of NK function by histones seemed, at least partly, to result from a toxic effect on effector cells, no longer able to kill target cells, a mechanism that did not occur for nucleosomes and DNA. However, after a 24-h incubation, histones altered neither NK activity nor cell viability (more than 98% cell viability) in PBMC. These results suggested a protective role of adherent cells (monocytes/macrophages) from histone toxicity. To test this possibility, histones and DNA (100 µg/ml) were added to 4-h ^{51}Cr tests with, as effector cells, a fixed number of PBL and an increasing number of adherent cells, isolated from the same PBMC suspension. The results showed a progressive disappearance of the NK inhibition induced by histones according to the increasing adherent cells/PBL ratio (Fig. 5). A significant protective effect of adherent cells ($P < 0.05$ versus PBL) appeared at a 0.125 adherent cells/PBL ratio. By contrast, the inhibition of NK-cell-mediated cytotoxicity was stable with DNA addition, irrespective of the proportion of adherent cells in the effector population (Fig. 5). In the same way, mononucleosome addition (10 µg/ml) inhibited the NK activity when PBL (depleted in monocytes) and PBMC (corresponding to a 0.2–0.5 adherent cells/PBL ratio) were used as effector cell suspensions. Indeed, the cytotoxic potentials of PBMC and PBL (expressed as NK cytotoxic percentages for an E:T ratio of 25), decreased respectively from 38±6% and 39±8% in controls to 21±4% and 20±7% in the presence of nucleosomes ($n = 5$; $P < 0.01$ versus controls).

Effect of mononucleosomes on NK-mediated lysis

As mentioned above, after a 24 h preincubation with mononucleosomes and washing, PBL showed unaltered NK activity, whereas nucleosomes added in a 4-h test strongly inhibited the NK lysis. This lack of effect could be due to a disappearance of nucleosomes from the medium and/or from the cell surface after 24 h, because of washing and/or catabolism and cellular endocytosis. To explore the ne-

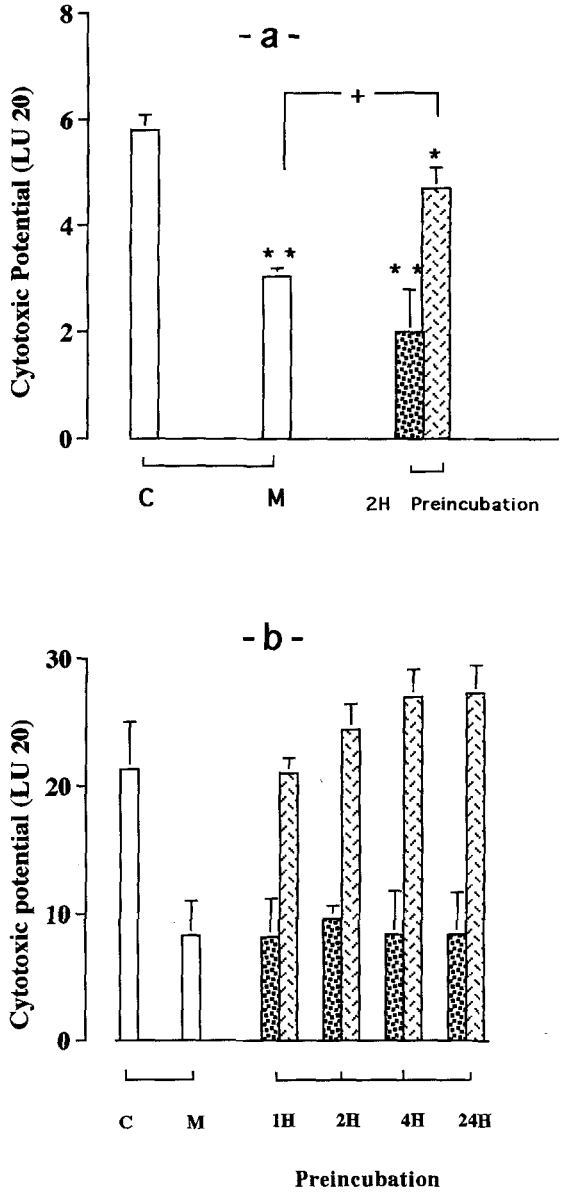

Fig. 6a, b. Effect of washing on the impairment of NK activity induced by mononucleosomes. NK cytotoxicity of a PBL suspension was assessed in a ^{51}Cr test using (**a**) naive effector cells and target cells preincubated for 2 h with nucleosomes or (**b**) nucleosome-preincubated effector cells (for 1, 2, 4 or 24 h) with naive target cells. Preincubated cells were washed (▨) or simply centrifuged after discarding the supernatant (▩) before the test. Cytotoxic potentials are expressed in $LU_{20}/10^6$ effector cells, as the mean ± SEM of three independent experiments. $^*P < 0.05$; $^{**}P < 0.01$ versus control (*C*); $^+P < 0.05$ versus M. *C* and *M* represent respectively PBL without nucleosomes and PBL with mononucleosomes added directly in the ^{51}Cr test

cessity for a direct presence of nucleosomes in the extracellular medium to induce an inhibition of NK activity, target cells and effector cells were preincubated with mononucleosomes (10 µg/ml) for respectively 2 h and 1, 2, 4 or 24 h, then washed or simply centrifuged, the supernatant was discarded before the ^{51}Cr test. A 2-h preincubation of target cells with nucleosomes allowed the NK activity inhibition ($P < 0.01$ versus controls), while washing of cells after the preincubation abolished almost totally the nucleosome-mediated inhibition (Fig. 6a). In the same way, washing of effector cells preincubated with nucleosomes suppressed the inhibition of NK activity (Fig. 6b). Indeed, cytotoxic potentials decreased from 21 ± 4 LU_{20} in controls to 8 ± 3 LU_{20} ($P < 0.01$) when nucleosomes were added in the chromium test, and to 8 ± 3, 10 ± 1, 8 ± 3 and 8 ± 3 LU_{20} with cells preincubated with nucleosomes for respectively 1 h, 2 h, 4 h and 24 h. When cells were washed after incubation, cytotoxic potentials ± SEM were: 21 ± 1, 24 ± 2, 27 ± 2 and 27 ± 2 LU_{20} after respectively 1 h, 2 h, 4 h and 24 h.

These results showed that nucleosomes had a reversible effect on either population, effector or target cells, and clearly inhibited the NK function only when present in the extracellular medium.

Discussion

This study shows that, in patients with cancer, the presence of high concentrations of nucleosomic DNA in plasmas is correlated with an inhibitory effect of plasma on the NK activity. A suppressive effect of plasma and serum from cancer patients on NK activity has been previously reported [5, 13, 20]. While indirect, our results suggest a role for nucleosomes in this suppressive effect. Indeed, in vitro, nucleosomes were found to inhibit the NK-mediated cytotoxicity in a dose-dependent manner. Further experiments are required to investigate whether this phenomenon plays a significant pathophysiological role in vivo. The effective concentration of nucleosomes in plasma (about 10- to 40-fold lower than the required nucleosome concentration in the culture medium), suggests that the physicochemical properties of circulating nucleosomes in plasma from cancer patients differed from those of CHO nucleosomes or/and that other constituents of plasma are involved in this phenomenon. It has been shown, for example, that low-density lipoproteins can decrease both NK- and T-cell-mediated cytotoxicity [30].

DNA and histones were also found to inhibit NK activity. However, the effects of histones, DNA and nucleosomes differed according to the effector cell population and are likely to be mediated by different mechanisms. The inhibitory effect of histones could be due to a subtoxic effect on NK cells, possibly related to their cationic properties. The protective role of adherent cells could be explained by an efficient uptake of histones by monocytes. Indeed, it has been previously reported that histones are present at the surface of human monocytes [12, 23]. The inhibitory effect of free DNA is likely to need T cells since it was not observed when $CD3^+$-depleted PBL were used. It could involve cytokine production by T cells such as interleukin-3 (IL-3) and IL-4, which are known to inhibit NK activity [19].

Nucleosomes, histones and DNA could exert their modulatory activity through different receptors or binding sites for the chromatin fragments [2, 11, 12, 14, 23]. In addition, it must be stressed that chromatin fragments released into the circulation from dead cells are likely to

consist mainly of nucleosomes. The fact that mononucleosomes (and oligonucleosomes, results not shown) significantly inhibited the NK cytotoxicity at concentrations 10- to 40-fold lower than those of DNA or histones alone argues in favour of a receptor for the nucleosomes on mononuclear cells, as previously suggested [11, 14]. Such a receptor might be present on effector NK cells but also on leukaemic cells since a 2 h preincubation of target cells with nucleosomes also resulted in an inhibition of NK cytotoxicity. However, the NK inhibition induced after incubation of nucleosomes with effector or target cells was totally or partially lost after washing. Thus, nucleosomes must be present directly at the cell surface to be effective. This suggests a low-affinity binding of nucleosomes to a potential receptor or a rapid catabolism by cell surface receptors or even removal of the binding site by washing.

In healthy subjects, cell death occurs constantly through a physiological mechanism of programmed cell death or apoptosis. In vivo, phagocytes recognize apoptotic cells and ingest them to protect tissues from the harmful consequences of their nuclear material [25]. A rise of cell death during drug treatment or disease evolution might induce the release into extracellular spaces of a large amount of nucleosomal structures that might have some clinical relevance and, more particularly, might have some effect on the immune system. Up to now, nucleosomes have been shown to play a role in polyclonal B lymphocyte activation, cytokine production and in the induction of anti-DNA antibodies [1, 11, 16]. The persistence of a significant amount of nucleosomal DNA in tissues could also modulate the NK function through a negative-feedback mechanism. This inhibition might be involved, at least partly, in the NK impairment observed in cancer [5, 15, 17, 20, 28] and lupus [4, 10, 32]. In addition, the anti cancer drugs that trigger the pathway of programmed cell death [27] could be responsible for the release of chromatin fragments from tumors and induce the drop in NK activity observed after chemotherapy [15, 31]. A similar effect might be involved in the in vitro anthracycline-induced resistance of target tumor cells to NK cell attack [3, 31].

In conclusion, the present data demonstrate that extracellular nucleosomes inhibit NK activity. They suggest that an imbalance between production and clearance of nucleosomes during the process of cell death could play a role in the impairment of NK function that characterizes various pathological situations such as systemic lupus erythematosus, AIDS and cancer.

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