Human neutrophils, activated by phorbol-myristate acetate (PMA), (A-neutrophils), were found to suppress lymphocytic killer (K) cell-mediated antibody-dependent cellular cytotoxicity (ADCC). Resting (R) neutrophils, ie, PMA-unstimulated cells, were completely ineffective. Suppression was optimal when A-neutrophils were added at the beginning of the ADCC assay. Furthermore, A-neutrophils were found to cause an approximately 80% reduction in the number of Raji target cell-bound lymphocytes. These data indicate that A-neutrophils inhibit K cell activity by interfering with the target cell recognition. A-neutrophils were capable of reducing the percentage of Fc receptor (FcR)-bearing lymphocytes with a half-time of 7.2 minutes, through a process preventable by the serine-protease inhibitors tosyl-lysine-chloromethyl ketone (TLCK) and lima bean trypsin inhibitor (LBTI). Conversely, A-neutrophils caused a very slow decrease in the amount of Raji cell-bound antibodies, as detected by the complement-mediated lytic assay. Thus, only lymphocyte FcR structures seem to be highly susceptible to neutrophil-derived TLCK- and LBTI-inhibitable proteases. Furthermore, supernatants from A-neutrophils were found to inhibit K cell ADCC and lymphocyte binding to Raji target cells. In addition, LBTI prevented the A-neutrophil-dependent and the supernatant-dependent inhibition of both K cell ADCC activity and lymphocyte-target cell conjugate formation. Together these data suggest that A-neutrophils suppress K cell function through a protease-mediated impairment of the FcR binding capacity. The results provide evidence that human neutrophils are endowed with mechanisms to regulate K cell ADCC activity.

**Neutrophil Activation**

Neutrophils, suspended in ice-cold RPMI–FCS at a concentration of 10^6/mL, were incubated (five minutes, 4°C) with 100 ng/mL phorbol-12-myristate-13-acetate (PMA) (Sigma Chemical Co, St Louis). Cells were then centrifuged (160 g, four minutes, 4°C), washed twice in 50-mL volumes of ice-cold RPMI, counted, resuspended at an appropriate concentration in ice-cold RPMI–FCS and kept at 4°C until used. Each cell suspension contained more than 95% viable cells, as evaluated by the ethidium bromide–fluorescein diacetate test. Preliminary experiments showed that this procedure provides neutrophils in the lag phase before superoxide production, as detected by the continuous assay of Cohen and Chovaniec. These PMA-treated cells will be referred to as “activated” neutrophils (A-neutrophils).

**Target Cells**

Lymphoblastoid cells (Raji), derived from Burkitt’s lymphoma, were used as target cells. The Raji cell line (kindly supplied by Professor G. Holm, Department of Clinical Immunology, Karolinska Institute, Huddinge, Sweden) was grown in suspension (RPMI–FCS) and subcultured every four to five days.

By Franco Dallegri, Franco Patrone, Guido Frumento, Alberto Ballestrero, and Carlo Sacchetti

**Down-Regulation of K Cell Activity by Neutrophils**

A subset of human lymphocytes is capable of destroying IgG-coated target cells through the well-known process of antibody-dependent cellular cytotoxicity (ADCC).\(^1\)\(^2\) Lymphocytes with such ability have receptors for the Fc portion of IgG molecules (FcR) and are operationally referred to as killer (K) cells. Because of the occurrence of humoral antibodies against a variety of cells in several disease states, K cells have been considered to be an important component of tissue-damaging immune responses. As for other steps of the immune network, it is conceivable that regulatory cells may control the levels of K cell cytotoxic reactivity. In this connection, monocytes and nonadherent cells, lacking easily detectable markers, have usually been found to act as “suppressor” cells.\(^3\)\(^4\) Here we provide the first evidence that neutrophils are endowed with a potential to exert an effective down-regulation of K cell activity by preventing target cell recognition.

**Materials and Methods**

**Cell Preparation**

Heparinized venous blood (heparin 10 U/mL, Liquemin, Roche, Milan, Italy) was obtained from healthy volunteers and from a previously described patient with chronic granulomatous disease (CGD), (patient 2).\(^6\)

Mononuclear cells, isolated by centrifugation (400 g, 30 minutes) on a Ficoll-Hypaque (Nyegaard & Co, Oslo) density gradient,\(^1\) were suspended in RPMI 1640 (Flow Laboratories, Ltd, Irvine, Scotland) supplemented with 10% heat-inactivated (56°C, 30 minutes) fetal calf serum (FCS), 2 mmol/L glutamine, streptomycin 100 µg/mL, and penicillin 100 U/mL. Adherent cells were then removed by incubating (one hour, 37°C) mononuclear cells in thin suspension layers (5 × 10⁶ cells/mL, 4 mL) on plastic surfaces (75-cm² flasks, Sterilin, Teddington, England). Nonadherent cells were collected as “lymphocytes,” counted, and resuspended in RPMI–FCS at appropriate concentrations. Only cell suspensions containing < 1% monocytes, as determined by α-naphthyl acetate esterase staining,\(^6\) were used.

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**Antiserum**

A New Zealand white rabbit was injected subcutaneously at multiple dorsal sites with 10⁶ Raji cells in 1 mL Hank's balanced salt solution (HBSS) (Flow Laboratories, Ltd). The injections were repeated eight times at four- to five-day intervals. Serum, harvested one week after the last injection, was heat-inactivated (56 °C, 30 minutes) and stored in aliquots at −70 °C.

**ADCC Assay**

ADCC was studied by a 5¹Cr-release method as previously referred. In brief, 10⁶ Raji cells were labeled with 100 to 200 μCi sodium chromate Cr 5¹ (Radiochemical Center, Amersham, England) by incubating for one hour at 37 °C. After washing, the cells (2 x 10⁶/mL) were sensitized (37 °C, 30 minutes) with an appropriate dilution of rabbit antiserum. Cells labeled with chromium 5¹ and sensitized Raji cells were subsequently washed twice and resuspended in RPMI–FCS at a concentration of 2 x 10⁶/mL. Target cells (2 x 10³) were mixed with lymphocytes at an effector–target ratio of 20:1. When required, appropriate concentrations of A-neutrophils or resting (R), ie, PMA-untreated, neutrophils were added to wells. The assays, performed in triplicate and in a final volume of 200 μL using round-bottom microtubes (Nunc, Roskilde, Denmark), were initiated by centrifugation (50 g, five minutes) at 22 °C. After four-hour incubation in humidified atmosphere of 95% air and 5% CO₂, the ¹Cr-release was determined in the cell-free supernatants. The percentage of cytotoxicity (% ¹Cr-release) was calculated according to the formula 100 x (E – S)/(T – S), where E is the cpm released in the presence of effector cells, T is the cpm released after lysing target cells with 5% Triton X-100 (Sigma Chemical Co), and S is the cpm spontaneously released by target cells incubated with medium alone (in each case ≈ 12%).

**Target Cell Binding Assay**

The assay was performed under the same conditions used for cytotoxicity tests. In brief, lymphocytes were mixed with sensitized Raji cells, in the presence or absence of A-neutrophils or R-neutrophils. After incubation (30 minutes, 37 °C), the number of target cell-bound lymphocytes was determined on Giemsa-stained smears.

**Detection of Fc Receptors**

Fc receptor-bearing lymphocytes were detected by a rosette technique as previously described, using ox erythrocytes, appropriately sensitized with purified rabbit IgG antibody, as indicator cells (EA₅₇). When tests were performed on cell populations containing A- or R-neutrophils, lymphocytes were prelabeled with fluorescein diacetate (FDA) (Sigma Chemical Co). In brief, lymphocytes were incubated (15 minutes, 22 °C) with 2 ng/mL FDA in RPMI and subsequently washed twice before using. Detection of Fc receptor-bearing cells was performed by mixing cell suspension (100 μL) with an equal volume of 1% suspension of EA₅₇ in disposable plastic tubes. The tubes were subsequently centrifuged at 150 g for five minutes and incubated at 4 °C for 30 minutes. After resuspension, the percentage of rosette-forming lymphocytes (RFL) was determined by counting a minimum of 200 lymphocytes.

**Estimation of Raji Cell-Bound Antibodies**

The amount of antibodies on the sensitized Raji cells was judged by the intensity of 1¹Cr-labeled target cell lysis by complement. In brief, sensitized and labeled Raji cells, incubated with and without A- or R-neutrophils under experimental conditions identical to ADCC assays, were resuspended before treatment with rabbit complement (Peel-Freeze Biologicals, Rogers, Ark) and further incubated for 45 minutes at 37 °C. Control experiments were performed by using heat-inactivated (56 °C, 45 minutes) rabbit complement and unsensitized Raji cells. After sample centrifugation (100 g, five minutes), the ¹Cr-release was detected in the cell-free supernatants.

**Special Materials**

PMA, (Sigma Chemical Co), stored at −20 °C as stock solution of 2 mg/mL in dimethyl sulfoxide (DMSO, C. Erba, Milan, Italy) was diluted in medium before use. Superoxide dismutase (SOD, type I, bovine blood, E.C.1.15.1.1.), catalase (bovine liver, E.C.1.11.1.6), lima bean trypsin inhibitor (LBTI) and Na-p-tosyl-L-lysine-chloromethyl ketone (TLCK) were purchased from Sigma Chemical Co. LBTI was extensively dialyzed against RPMI before use.

**RESULTS**

When added to the K cell ADCC system (lymphocyte-Raji ratio 20:1), A-neutrophils inhibited the target cell lysis in a dose-dependent manner (Fig 1). R-neutrophils had no inhibitory effect (Fig 1). As shown in Table 1, sonicated A-neutrophils did not affect K cell activity; therefore, ADCC suppression by intact A-neutrophils does not involve carryover of inhibitory doses of PMA into test wells by A cells themselves (PMA was found to cause, per se, a dose-dependent inhibition of K cell activity, data not shown). Under the experimental conditions employed, A-neutrophils do not behave like active cytotoxic cells against sensitized Raji cells or lymphocytes. This is suggested by different lines of evidence. First, A-neutrophils required at least a two-log greater amount of antitarget antibody than did lymphocytes to kill Raji cells efficiently (Table 1). Second, lymphocytes labeled with chromium 5¹, cocultured with A-neutrophils and unlabeled sensitized target cells as for ADCC assays, had a percentage of ¹Cr-release of −0.9 ± 2.3, 1 SD (n = 3). Together, these data indicate that: (a) neutrophils are endowed with a potential to suppress K cell activity; (b) cell activation by PMA is required to
DOWN-REGULATION OF K CELL ADCC BY PMN

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expressed as i ± 1 SD. n

subsequently the cells were gently resuspended before the addi-

tion of medium (0) or A-neutrophils ( ● ).

plus Raji cells were centrifuged and incubated (30 minutes, 4 °C); neutrophils added at the beginning of the assay. (B) Lymphocytes ( ○ ) and A-neutrophils (●) were added before or after lymphocyte-Raji cell conjugate formation. Therefore, suppression occurs mainly at the binding step of the K cell-mediated ADCC process. To gain insight into the mechanisms underlying this event, experiments were designed to test the effect of A-neutrophils on the lymphocyte FcR binding capacity and on the amount of Raji cell-bound antibodies. As depicted in Fig 3, A-neutrophils caused a progressive reduction in the percentage

trigger the neutrophil inhibitory function; (c) A-

neutrophils interfere with the expression of K cell activity without affecting lymphocyte "viability."

It is well documented that K cell-mediated ADCC involves at least two separable processes: binding and lysis.1,2,15 Thus, A-neutrophils might suppress K cell activity by interfering at one or both of these steps. To test these possibilities, A-neutrophils were added to the ADCC system before or after lymphocyte–Raji cell conjugate formation. As depicted in Fig 2, the addition of A-neutrophils after the effector–target binding almost completely prevented the inhibition of K cell activity. Therefore, suppression occurs mainly at the binding step of the K cell-mediated ADCC process. To gain insight into the mechanisms underlying this event, experiments were carried out by the target cell binding microscopic assay, performed by incubating lymphocytes with sensitized Raji cells in the presence and in the absence of A-neutrophils or R-neutrophils. As shown in Table 2, A-neutrophils added to this assay caused a significant reduction in the number of target cell-bound lymphocytes. R-neutrophils were ineffective (Table 2). Therefore, A-neutrophils are capable of preventing an optimal lymphocyte–Raji cell conjugate formation. Because identical experiments performed at 4 °C showed that lymphocytes bound equally to Raji cells (P > .5) in the presence and in the absence of A-neutrophils, a simple steric interference can be ruled out. In addition, attachment of A-neutrophils to Raji cells was always absent (< 5 Raji cell-bound A-neutrophils per 100 Raji cells): consequently, competition of A-neutrophils with K cells for binding sites on the target cell surface does not occur in our system. These results indicate that suppression of K cell activity by A-neutrophils involves an impaired target cell recognition by lymphocyte effector cells.

Binding of sensitized targets by K cells requires an interaction between K cell FcR and its ligand at the target cell level. Thus, the A-neutrophil–dependent suppression might be related to a reduced availability of lymphocyte or of Raji cell recognition structures. To investigate this possibility, experiments were designed to test the effect of A-neutrophils on the lymphocyte FcR binding capacity and on the amount of Raji cell-bound antibodies. As depicted in Fig 3, A-neutrophils caused a progressive reduction in the percentage

Table 1. Inhibition of Lymphocyte-ADCC by A-Neutrophils

<table>
<thead>
<tr>
<th>Cells Added to Raji Targets: *</th>
<th>Antiserum † (%)</th>
<th>51Cr-Release (%) (G = 1 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>0.1</td>
<td>48.2 ± 6.5 (n = 6)</td>
</tr>
<tr>
<td>Lymphocytes + A-neutrophils</td>
<td>0.1</td>
<td>11.2 ± 6.0 (6)</td>
</tr>
<tr>
<td>Lymphocytes + A-neutrophils ‡</td>
<td>0.1</td>
<td>52.5 ± 5.0 (5)</td>
</tr>
<tr>
<td>A-neutrophils</td>
<td>0.1</td>
<td>3.7 ± 0.7 (3)</td>
</tr>
<tr>
<td>A-neutrophils</td>
<td>1.0</td>
<td>2.0 ± 0.5 (5)</td>
</tr>
<tr>
<td>A-neutrophils</td>
<td>10.0</td>
<td>17.8 ± 3.0 (6)</td>
</tr>
<tr>
<td>A-neutrophils</td>
<td>20.0</td>
<td>74.5 ± 5.5 (6)</td>
</tr>
</tbody>
</table>

* Raji cells, 2 x 10⁶; lymphocytes, 4 x 10⁶; A-neutrophils, 8 x 10⁶. † Percentage of antiserum used to sensitize Raji cells labeled with chromium 51. ‡ Sonicated A-neutrophils: neutrophils were pretreated at 4 °C with PMA (see Materials and Methods) and sonicated at 4 °C before use.

Fig 2. Suppression of K cell ADCC activity by A-neutrophils added before or after lymphocyte–Raji cell conjugate formation. (A) ADCC in the absence (●) and in the presence (●) of A-neutrophils added at the beginning of the assay. (B) Lymphocytes plus Raji cells were centrifuged and incubated (30 minutes, 4 °C); subsequently the cells were gently resuspended before the addition of medium (●) or A-neutrophils (●). Preliminary experiments showed that 30-minute incubation allowed optimal lymphocyte–Raji cell binding. For cell ratios, see Table 1 legend; results are expressed as i ± 1 SD, n = 4.

Table 2. Inhibition of Lymphocyte-Raji Cell Binding by A-Neutrophils

<table>
<thead>
<tr>
<th>Cells Added to Raji Cells: *</th>
<th>Raji Cell-bound Lymphocytes † (G = 1 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>359.0 ± 53.3</td>
</tr>
<tr>
<td>Lymphocytes + A-neutrophils</td>
<td>94.5 ± 11.4</td>
</tr>
<tr>
<td>Lymphocytes + R-neutrophils</td>
<td>331.0 ± 56.3</td>
</tr>
</tbody>
</table>

* Raji cells sensitized with 0.1% antiserum, 2 x 10⁶; lymphocytes, 4 x 10⁶; neutrophils, 8 x 10⁶. † Number of Raji cell-bound lymphocytes/100 Raji cells; results of four experiments.

Fig 3. Effect of A-neutrophils (●, □) and R-neutrophils (○, □) on lymphocyte FcR (left) and on Raji cell-bound antibodies (right). Each assay, including ratios, was performed under the same experimental conditions used for ADCC tests. Results are expressed as percentage of time zero values (ordinates) and depicted as i ± 1 SD of four experiments. Time zero EA-RFL incubated with A-neutrophils: log y = −0.0467 × + 2.036, r = −.997, P < .001. The amount of Raji cell-bound antibodies was judged by the intensity of complement-mediated ⁵¹Cr-release (see Materials and Methods): time zero complement-mediated ⁵¹Cr-release ranged from 68% to 80%.
of the EA₀ rosette-forming lymphocytes with a half-time of 7.2 minutes; rosettes were virtually absent after 30 minutes. Conversely, 30 minutes were required to obtain a percentage decrease of 26.3 ± 3.8, 1 SD in the amount of Raji cell-bound antibodies, as detected by the complement-mediated lytic assay (Fig 3). Thus, A-neutrophils act on both lymphocyte effector cells and Raji target cells; the antibodies are, however, lost by target cells at a relatively slow rate and, consequently, the impaired K cell binding in our ADCC system can be more conceivably explained by the fast reduction in the percentage of FcR-bearing lymphocytes. Unfortunately, further experiments with lymphocytes and with Raji cells, reisolated after interaction with A-neutrophils, could not be appropriately performed because of the impossibility of obtaining cell populations free of contaminating A-neutrophils. This probably reflects a PMA-induced cell-density heterogeneity, already described for in vitro activated neutrophils.¹⁶

Our conclusion that A-neutrophils suppress K cell ADCC activity through an impaired target cell recognition, probably related to a loss of K cell FcR binding capacity, suggests a possible involvement of neutrophil-derived proteases in the phenomenon. Indeed, lymphocyte FcRs are susceptible to proteolysis.¹⁷ In particular, it has been shown that these surface structures can be completely removed by pronase within 30 minutes,¹⁸ a time that would be consistent with our findings. In this connection, the A-neutrophil-dependent inhibition of lymphocyte EA₀-rosetting capacity was completely prevented by the serine-esterase inhibitor TLCK and partially prevented by the macromolecular antiprotease LBTI (Table 3). Thus, it does not seem unreasonable to consider lymphocyte FcRs as structures highly susceptible to neutrophil-derived proteases. Consequently, we studied the effects of serine-protease inhibitors on the A-neutrophil-dependent suppression of K cell ADCC and of lymphocyte binding to Raji target cells. Because TLCK directly inhibited K cell function¹⁹ (data not shown), LBTI only could be tested. LBTI reduced the A-neutrophil-mediated suppression of Raji cell lysis and of Raji cell binding by more than 60% and 50%, respectively (Table 4). These results suggest a requirement for neutrophil-derived protease(s) in the observed inhibition of K cell activity. A possible role of an A-neutrophil-dependent oxidant stress can be excluded; in fact, scavengers of oxygen metabolites (SOD, catalase) completely failed to prevent the suppression of K cell activity (Table 4), and PMA-treated CGD neutrophils were as effective in inhibiting K cell ADCC as normal A-neutrophils tested in a parallel assay (Fig 4).

A series of experiments to determine whether or not an inhibitory activity could be demonstrated in the supernatants from A-neutrophils was undertaken. A-neutrophils (1.6 x 10⁶/mL) were incubated in RPMI for three hours at 37 °C: the supernatants recovered from these suspensions significantly inhibited both the lymphocyte-mediated ADCC and the lymphocyte binding to Raji cells (Table 5). Supernatants from R-neutrophils had no inhibitory activity (Table 5). Furthermore, the capacity of the supernatant from A-neutrophils to suppress lymphocyte target cell binding and lysis was reduced approximately 60% by LBTI (Table 5). Thus, A-neutrophils release a soluble factor or factors reacting with the macromolecular antiprotease LBTI, that is capable of efficiently mediating an impaired target cell recognition and killing by K cells.

**DISCUSSION**

Our results indicate that A-neutrophils are capable of inhibiting K cell ADCC activity efficiently. Two main phenomena underlie this observation. First, considerably greater amounts of target-bound antibody are needed to support neutrophil ADCC as compared to lymphocyte ADCC²⁰,²¹; under our experimental conditions, neutrophils required approximately two-log greater amounts of antitarget cell serum than did lymphocytes. Second, A-neutrophils are capable of destroying target cells by using oxidative means²²-²⁴; nevertheless, some kinds of target cells, endowed with optimal antioxidant defenses, are resistant in this cytotoxicity system²⁵ and, like Raji cells, may require the presence of high doses of iodide to be killed.²⁵ Consequently, it is not surprising that, under the experimental conditions used, A-neutrophils do not behave like active killer cells against both Raji cells and lymphocytes. This is documented by two findings:

| Table 3. Effect of Serine-Protease Inhibitors on EA₀-rosette-forming Lymphocytes in the Presence of A-Neutrophils |
|---------------------------------|-----------------|
| Lymphocytes Incubated With: *   | EA₀-RFL (%)     |
| A-neutrophils                   | 2.0 ± 1.4 (n = 6) |
| A-neutrophils + TLCK 0.2 mmol/L| 2.1 ± 2.1 (3)    |
| A-neutrophils + LBTI 2.5 mg/mL  | 1.3 ± 2.5 (3)†  |
| Medium alone                    | 2.1 ± 3.7 (6)   |
| TLCK 0.2 mmol/L                 | 2.3 ± 2.6 (3)   |
| LBTI 2.5 mg/mL                  | 2.1 ± 3.0 (3)   |

RFL, rosette-forming lymphocytes; TLCK, Na-p-tosyl-L-lysine-chloromethyl ketone; LBTI, lima bean trypsin inhibitor.

*Lympocytes (2 x 10⁶), with and without A-neutrophils (4 x 10⁹), were incubated for 30 minutes at 37 °C in the presence of the various agents before EA₀ rosette assay.

†P < .001 when compared to the result obtained with lymphocytes + A-neutrophils alone.
(a) virtually no binding and lysis of Raji cells by A-neutrophils could be observed when these targets were appropriately sensitized for K cell ADCC; and (b) no release of radioactivity from lymphocytes labeled with chromium 51 was detected when they were tested under conditions identical to the A-neutrophil-inhibitable K cell ADCC assay.

It is conceivable that K cell ADCC involves a sequence of processes: lymphocyte binding to IgG sensitized target cells, followed by postbinding generation and transduction of lytic signal or signals to cell effector systems, activation, and expression of the lytic armamentarium itself. In our model system, when A-neutrophils were added after lymphocyte-target conjugate formation, only an approximate 25% inhibition of K cell ADCC could be detected. Conversely, addition of A-neutrophils at the beginning of ADCC assays produced approximately 80% inhibition. These findings indicate that A-neutrophils interfere mainly with the first step or steps of the K cell ADCC process to produce suppression. In this regard, the microscopic binding assay showed that A-neutrophils cause 70% to 80% reduction in the number of target-bound lymphocytes. Thus, K cell ADCC suppression by A-neutrophils, added at the beginning of the assay, involves an impaired target cell recognition by lymphocyte effector cells. The impaired target cell recognition can also account for the slight suppression observed when A-neutrophils are added to preformed effector–target conjugates, because of its possible interference with K cell recycling capacity. This is an hypothesis, however, and requires an experimental demonstration. In this connection, prostaglandins have been reported to inhibit K cell ADCC by acting at postbinding steps26,27; results obtained in our own laboratory showed that indomethacin did not affect suppression of K cell activity (unpublished observations), ruling out the possible involvement of prostaglandins even to explain the minor inhibitory effect of A-neutrophils added after lymphocyte–target cell conjugate formation.

The fine mechanisms leading to the impaired target cell recognition by K cells in the presence of A-neutrophils are not completely understood. At present, hypotheses on this topic are difficult to test experimentally, mainly because the complexity of our system, which involves two active “effector” cells, ie,

<table>
<thead>
<tr>
<th>Lymphocytes Incubated With:</th>
<th>Raj Cell Lysis (% 11C-Release)</th>
<th>Raj Cell-bound Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium alone</td>
<td>47.6 ± 7.6 (n = 6)</td>
<td>311.7 ± 69.9 (3)</td>
</tr>
<tr>
<td>A-neutrophils</td>
<td>14.1 ± 5.0 (6)</td>
<td>66.3 ± 11.8 (3)</td>
</tr>
<tr>
<td>A-neutrophils + LBTI</td>
<td>35.2 ± 3.4 (3)</td>
<td>178.0 ± 21.9 (3)</td>
</tr>
<tr>
<td>A-neutrophils + SOD</td>
<td>13.0 ± 3.7 (3)</td>
<td>ND</td>
</tr>
<tr>
<td>A-neutrophils + catalase</td>
<td>10.3 ± 1.9 (3)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Results are expressed as x ± 1 SD of (n) experiments; ND, not done; A-neutrophils + LBTI v A-neutrophils, P < .001 for both the Raj cell lysis and the Raj cell binding.

*LBTI, 2.5 mg/mL; SOD, 300 U/mL; catalase, 4,000 U/mL. None of these agents inhibited lymphocyte ADCC.

†Lymphocyte-Raji cell ratio 20:1; A-neutrophil-lymphocyte ratio 2:1; Raji cells, 2 × 10⁶.

‡Number of Raji cell bound lymphocytes per 100 Raji cells (see Table 2 legend).

---

**Table 5. Effect of Supernatants From A-Neutrophils on Lymphocyte-ADCC and Lymphocyte-Raji Cell Binding**

<table>
<thead>
<tr>
<th>Supernatant</th>
<th>Raj Cell Lysis (% 11C-Release)</th>
<th>Raj Cell-bound Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI</td>
<td>39.4 ± 3.9</td>
<td>324.0 ± 42.0</td>
</tr>
<tr>
<td>A-neutrophils supernatant</td>
<td>13.9 ± 2.5</td>
<td>116.7 ± 30.2</td>
</tr>
<tr>
<td>A-neutrophils + LBTI supernatant</td>
<td>29.4 ± 1.6</td>
<td>234.3 ± 40.7</td>
</tr>
<tr>
<td>R-neutrophils supernatant</td>
<td>41.3 ± 3.0</td>
<td>317.0 ± 57.0</td>
</tr>
<tr>
<td>R-neutrophils + LBTI supernatant</td>
<td>40.0 ± 1.8</td>
<td>ND</td>
</tr>
</tbody>
</table>

Results are expressed as x ± 1 SD of three experiments; ND, not done; Raj cell lysis, C v B P < .001; Raj cell-bound lymphocytes, C v B P < .02.

*Lymphocytes were incubated (30 minutes, 37 °C) with 50% (vol/vol) supernatant or RPMI in the presence or in the absence of 2.5 mg/mL LBTI and then tested for ADCC and binding capacity. The supernatants were recovered from suspensions of A-neutrophils or R-neutrophils (1.6 × 10⁶ cells/mL), incubated at 37 °C for three hours in RPMI.

†Lymphocyte-Raji cell ratio 20:1; Raji cells, 2 × 10⁶.

‡Number of Raji cell-bound lymphocytes per 100 Raji cells (see Table 2 legend).
A-neutrophils and K cells, allows the addition of few probes to interfere selectively with the A-neutrophil suppressive activity alone. Certainly, oxygen metabolites released by A-neutrophils are not involved, as indicated by the fact that PMA-treated CGD neutrophils as well as normal A-neutrophils in the presence of oxygen metabolite scavengers efficiently inhibited K cell activity. Our finding that A-neutrophils interfere with lymphocyte EA-r-rosetting capacity through a process completely preventable by TLCK and partially preventable by LTBI, coupled with the well-known lymphocyte FeR susceptibility to proteolysis by pro-nase (and data not shown), is consistent with the involvement of a neutrophil-derived serine-protease or proteases as mediator of the impaired lymphocyte FeR binding capacity. Consequently, A-neutrophils could act in a similar manner to prevent target cell recognition by K cells during the ADCC process. Two different findings support this conclusion. First, the macromolecular antiprotease LTBI consistently prevented the A-neutrophil-dependent inhibition of both K cell ADCC activity and lymphocyte-target cell conjugate formation. Second, supernatants from A-neutrophils inhibited both the K cell ADCC and the lymphocyte binding to Raji target cells through a process preventable by LTBI itself. In addition, preliminary experiments performed in our own laboratory showed that the inhibitory factor (or factors) released by A-neutrophils was nondialyzable and was destroyed by heating for 30 minutes at 63 °C but not at 56 °C (unpublished observations). This is also consistent with the neutrophil protease-release concept. In this connection, it is of note that a protease (or proteases) would become activated or available after neutrophil PMA-triggering only. Indeed, when neutrophils were pretreated with TLCK, which irreversibly binds to the active sites of isolated serine-proteases, and subsequently challenged with PMA, they were still capable of inhibiting K cell ADCC (unpublished observations).

Seaman and co-workers and Kay and Smith have recently demonstrated that neutrophils can suppress lymphocyte K cell activity. Our data provide evidence that neutrophils are also endowed with mechanisms capable of modulating K cell activity by preventing target cell recognition. Additional studies are required to define the suppressive capacity of in vivo activated neutrophils and to characterize the biochemical pathways underlying the activation and the expression of this previously unrecognized neutrophil function further. However, it is tempting to speculate that the neutrophil suppressive capacity might be envisaged as an immunoregulatory device to limit the effects of the K cell cytolytic potential against antibody-coated tissue cells, for example, during autoimmune diseases, viral infections, and allografts.

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