Involvement of Tumor Necrosis Factor in Human Granulocyte-Mediated Killing of WEHI 164 Cells

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Abstract

Human polymorphonuclear leukocytes (PMNLs) kill WEHI 164 clone 13 cells in an 18-hour ⁵¹Cr release assay. Antibody to human tumor necrosis factor (TNF) blocks the lysis of targets mediated by human granulocytes. PMNLs triggered by sensitive targets not only displayed cytotoxic activity, but also released a soluble factor capable of selectively lysing WEHI 164 cells. The killing of these cells by supernatants of triggered granulocytes was totally inhibited by anti-TNF antibody. These experiments suggest that the killing of WEHI 164 sarcoma cells by human PMNLs involves TNF or TNF-like molecules.

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The cytotoxic effector cells of the immune system include several cell types. Cytotoxic T lymphocytes, lymphokine-activated killer cells, natural killer cells, monocyte/macrophage and natural cytotoxic (NC) cells are all capable of killing various target cells. The NC cells [Stutman et al., 1979] require 16–22 h to lyse their target, the slow killing of NC target cells is predominantly mediated by tumor necrosis factor (TNF) [Ortaldo et al., 1986]. Human lymphocytes can lyse the prototype of murine NC target cells WEHI 164. This human antitumor target cell activity is usually referred to as monocyte-mediated lysis and is preferentially mediated by TNF [Bersani et al., 1986]. In contrast with studies utilizing human mononuclear effector cells and murine targets, our studies represent the first demonstration that human polymorphonuclear leukocytes (PMNLs) are able to lyse WEHI 164 cells.

Figure 1 shows the cytotoxic activity of human PMNLs against WEHI 164 sarcoma cells in a 4-h and an 18-hour ⁵¹Cr release assay. Human PMNLs were purified (98%) from heparinized blood of healthy volunteers by dextran sedimentation, followed by Fi-coll-Uromiro (Bracco, Italy) centrifugation. The viability found by trypan blue exclusion was 98%. Cytotoxic assays and cell cultures were performed in RPMI 1640 supplemented with 2 mM glutamine and 10% fetal calf serum (Gibco). A considerable cytotoxicity was exerted by human PMNLs after the 18-hour incubation, but not after a 4-hour incubation period. Data are the means of the results of 15 independent experiments. No cytotoxicity was observed when some different cell lines, such as K-562, Hep-2 or YAC-1, were used [data not shown].

Moreover, granulocyte supernatants obtained by culturing human peripheral PMNLs together with WEHI 164 cells were effective in lysing ⁵¹Cr-labeled targets in a dilution of 1:2 (15.6 ± 4.6; n = 3). We presume that direct contact between effector granulocytes and target cells is essential for the production of lytic molecules, because supernatants of control PMNLs failed to lyse WEHI 164 cells.

The supernatant of triggered granulocytes lysed WEHI 164 clone 13 cells selectively, as other cells (K-562, HeP-2 and YAC-1) were not killed by granulo-cyte-released factor [data not shown].

As reported elsewhere [Espevik and Nissen-Meyer, 1986], WEHI 164 clone 13 is extremely sensitive to TNF, even without Actinomycin D treatment to potentiate the sensitivity. Therefore, we examined whether TNF is involved in the granulocyte-mediated cytotoxicity. For this purpose, we added antihuman monoclonal TNF antibody (Genentech) to the assay system. Sufficient antihuman TNF antibody to block

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Fig. 1. Specific lysis of WEHI 164 clone 13 cells by human PMNLs in 18 h (O) and in 4 h (•) ^{51}Cr release assay. Data are means $\pm$ SD of the results of 15 experiments.

Table 1. Inhibition of killing of WEHI 164 cells by anti-TNF antibodies

a Obtained by incubating 5×10^6 PMNL with 2×10^5 WEHI 164 in culture medium for 24 h.

b Supernatant was incubated with antibody for 30 min before addition of ^{51}Cr -labeled target cells.

103 or 5×10^2 U of recombinant human TNF was added to each cell assay at a final dilution of 1:1,000 or 1:1,200, respectively, at the beginning of the reaction. The table shows that the 1:1,000 dilution of antibody markedly reduced the granulocyte-mediated lysis at all effector:target ratios. Addition of a lower amount of antibody (1:2,000 dilution) to the culture produced less blocking. In the representative experiment shown in table 1, granulocyte supernatant obtained after a 24-hour incubation with WEHI 164 cells exerted a 14.5%

cytotoxicity against ⁵¹Cr-labeled target cells (in 1:2 dilution). The addition of anti-TNF antibody in 1:2,000 dilution abolished the cytotoxicity.

In our experiments, the inhibitory effect of anti-TNF antibody clearly demonstrated that TNF was actually involved in the granulocyte-mediated killing of WEHI 164 cells. It was more effective in neutralizing the toxic supernatant of the granulocytes, and it partially blocked the cytotoxicity when it was applied directly to the effector and target cells. It has recently been reported that TNF-sensitive cells are highly sensitive to oxidant injury [Zimmerman et al., 1989]. Therefore, not only TNF, but also other products of granulocytes, i.e. reactive oxygen species, might be involved in the cytotoxicity against WEHI cells.

PMNL-specific triggering signal like phorbolmyristic acetate (TPA) failed to induce cytotoxin (TNF) release. It is interesting, however, that in our pilot experiments incubation of PMNLs with heat-killed *Staphylococcus aureus* or with *Candida albicans* for 18 h led to the production of cytotoxic supernatants against WEHI 164 cells. The role of microorganisms in triggering of PMNLs to produce cytotoxin is under investigation in our laboratory.

The cytotoxicity of PMNLs against WEHI 164 cells seems to be highly specific, as neither normal cells like nonadherent PBL nor other cell lines such as K-562, YAC-1 or the T cell line MOLT-4 could trigger granulocytes to be cytotoxic. Moreover, the cytotoxicity of granulocytes was observed only against the extremely TNF-sensitive WEHI 164 cells, and L-929 cells which can be regarded as a TNF-sensitive cell line as well were not lysed by granulocytes at least without Actinomycin D. These results might be explained by the relatively low amount of TNF produced by human granulocytes, and very likely a highly sensitive test system has to be applied to detect TNF. This hypothesis is supported by our recent findings that an extremely high amount of LPS (*Escheri-*

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chia coli 0 128 B 12 L-2755 Sigma) was necessary (50–100 µg/ml) for a slight enhancement of cytotoxicity. At lower concentrations (0.1–25 µg/ml) of LPS, the LPS-treated granulocytes neither exerted higher cytotoxicity nor produced cytotoxic supernatant. These results suggest that the mechanism of TNF release from monocytes and PMNLs may be different. The present study provides evidence that NC activity can be mediated by human PMNLs. In addition, the triggering of granulocytes by target cells causes the release of a soluble mediator that lyses WEHI cells, but the factor is not present in nontriggered cell supernatants (control supernatants). The presence of TNF in human mast cells has recently been described [Steffen et al., 1989]. The cytotoxin from PMNLs of ascitic fluid in mice is suspected to be similar or identical to TNF [Yamazaki et al., 1989]. TNF has been found in NK cells [Degliantoni et al., 1985] and fibroblasts [Rubin et al., 1985]. Therefore, TNF is not a specific product of monocytes/macrophages. PMNLs can probably also produce TNF, and we conclude that the killing of WEHI 164 cells by human granulocytes involves TNF or a TNF-like molecule. Besides their antimicrobial capacity, therefore, granulocytes may play a useful function in tumor rejection, TNF production and immune surveillance, particularly if they are related to NC cells. The mechanism by which granulocytes are stimulated to release TNF is currently being investigated.

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References

- Bersani, L.; Colotta, F.; Mantovani, A.: Involvement of tumor necrosis factor in monocyte-mediated rapid killing of actinomy-cin D-pretreated WEHI 164 sarcoma cells. *Immunology* 59:323 (1986).
- Degliantoni, G.; Murphy, M.; Kobayashi, M.; Francis, M.K.; Perussia, B.; Trinchieri, G.: NK cell-derived hematopoietic colony-inhibiting activity and NK cytotoxic factor relationship with tumor necrosis factor and synergism with immune interferon. *J. exp. Med.* 162: 1512(1985).
- Espevik, T; Nissen-Meyer, J.: A highly sensitive cell line WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. *J. immunol. Methods* 95: 99 (1986).
- Ortaldo, J.R.; Mason, L.H.; Mathieson, B.J.: Mediation of mouse natural cytotoxic activity by tumor necrosis factor. *Nature* 321: 700(1986).
- Rubin, B.Y.; Anderson, S.L.; Sullivan, S.A.; Williamson, B.D.; Carswell, E.A.; Old, L.J.: Nonhematopoietic cells selected for resistance to tumor necrosis factor produce tumor necrosis factor. *J. exp. Med.* 164: 1350(1986).
- Steffen, M.; Abboud, M.; Potter, G.K.; Young, Y.P.; Moore, M.A.S.: Presence of tumor necrosis factor or related factor in human basophil/mast cells. *Immunology* 66: 445 (1989).
- Stutman, O.; Paige, O.J.; Figarella, E.F.: Natural cytotoxic cells against solid tumors in mice. I. Strain and age distribution and target cell susceptibility. *J. Immun.* 121: 1919(1979).
- Yamazaki, M.; Ikenami, M.; Sugiyama, T: Cytotoxin from poly-morphonuclear leukocytes and inflammatory ascitic fluids. *Br. J. Cancer* 59: 353(1989).
- Zimmerman, R.J.; Marafmo, B.J.; Chan, A., Jr.; Landre, Ph.; Win-kelhake, J.L.: The role of oxidant injury in tumor cell sensitivity to recombinant human tumor necrosis factor in vivo. *J. Immun.* 142: 1405(1989).