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CONCISE REPORT

# Correlation Between the Stimulation of Human Neutrophil Function by Monoclonal Antibody and by Colony-Stimulating Factor

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**Purified human neutrophils from 48 individuals were tested for their capacity to kill antibody-coated target cells in vitro in the absence or presence of stimulating agents. The agents used to stimulate cytotoxic capacity were the monoclonal antibody (MAb) WEM-G1, colony-stimulating factor (CSF-$\alpha$), or mononuclear cell supernatant (MNC-SN). There existed an heterogeneity among the neutrophils of different individuals in the capacity to kill target cells both in the unstimulated ("resting") or the stimulated state. A positive correlation was found between the ability of neutrophils to kill in the "resting" state and their capacity to be stimulated by MAb WEM-G1, CSF-$\alpha$, or MNC-SN. Furthermore, a strong positive correlation in the ability of neutrophils to be stimulated by the MAb WEM-G1 and either CSF-$\alpha$ ($r$ = .76) or MNC-SN ($r$ = .68), as well as between CSF-$\alpha$ and MNC-SN ($r$ = .79) was demonstrated. No correlation was seen, however, between stimulation of neutrophil function in vitro and total blood leukocyte counts, neutrophil counts, monocyte counts, or intensity of binding of MAb WEM-G1. The observation that neutrophils respond to a similar extent to different types of stimulators, such as cytokines (CSF-$\alpha$ and MNC-SN) and MAb, suggests that these two factors may be operating through a common mechanism and the degree of stimulation may reflect an intrinsic responsiveness of neutrophils that differs among individuals. Our results also suggest a potential clinical use of WEM-G1 in measuring neutrophil functional capacity in vitro and predicting the capacity to respond to CSF-like cytokines.**
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RECENT experiments have shown that some functions of human granulocytes are stimulated by cell-derived factors. For example, factors similar to, and in some cases identical to, colony-stimulating factors (CSF) enhance the killing of tumor[1,2] and fungal[2] targets by human neutrophils and the killing of parasite targets by human eosinophils.[3,4] It is likely that these factors act in vivo mainly at local sites of inflammation[5] where they are secreted by mononuclear cells.[2,6]

A marked heterogeneity in the capacity of individual's granulocytes to respond to these factors[2,7] has also been observed. The basis for this heterogeneity is not known but it could depend either on the number of receptors for activating factors or on the responsiveness of the cell to these factors. One approach to the understanding of such heterogeneity makes use of a recently developed monoclonal antibody (MAb), WEM-G1, which also stimulates human neutrophil function, as assessed by antibody-dependent cytotoxicity of tumor targets.[8] The stimulation by MAb does not appear to involve CSF receptors[9] and the MAb had no CSF-like or CSF-blocking effect in colony-forming assays (G. Begley, Walter & Eliza Hall Institute, personal communication, 1985). Thus, two agents, one a cell-derived regulator and the other a MAb, had similar influences on human neutrophil function. These observations allowed us to ask whether the heterogeneity in the response of neutrophils to CSF or CSF containing supernatants was also seen in their response to MAb.

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## MATERIALS AND METHODS

*Patient selection.* Twenty milliliters of blood was taken from 50 individuals who were either normal laboratory workers (n = 20) or patients in the Royal Melbourne Hospital (n = 30). The patients either suffered from a septic infection or were examined postoperatively. Informed consent was obtained from all individuals before venisection. The following additional data concerning the subjects were also collected: full blood analysis, degree of binding of MAb to granulocytes, and capacity of mononuclear cells to make granulocyte-activating factors.

*Reagents.* CSF-$\alpha$ was obtained by fractionation of human placental conditioned medium over phenyl Sepharose[10] and was used at a concentration that was two- to fivefold maximal in the colony-forming assay. Mononuclear cell supernatant (MNC-SN) was obtained from a single individual as described previously.[2] A single batch of MAb WEM-G1, raised against human neutrophils, was used at a concentration that gave maximal binding in test cases.[8] All reagents were derived from a single batch and quantities sufficient for the testing of cells from four individuals were aliquotted and frozen. After thawing, the reagents were not re-used in these experiments.

*Statistics.* Correlation coefficients, arithmetic means, and standard errors were determined with a Hewlett-Packard (Palo Alto, Calif) pocket calculator. Comparison of normal and abnormal groups was performed on a VAX-11 computer and a BMDPIR multiple linear regression analysis program (BMPD Statz Software, Univ of Calif at Berkeley).

*Preparation of neutrophils.* Neutrophils were obtained by purification on a hypertonic Metrizamide (Nyegaard, Oslo) gradient after dextran sedimentation of red cells as described previously.[11] Fraction 22, consisting of 97.9% ± 2.3% (arithmetic mean ± SD) pure neutrophils, was used routinely; however, in 20 patients, fraction 23 was also found to yield nearly pure neutrophils (98% ± 2.7%). When this was the case, neutrophils from both fractions were pooled.

*Antibody-dependent cell-mediated cytotoxicity assay.* This assay has been described in detail elsewhere.[1] Briefly, the amount of $^{51}$Cr released from trinitrophenyl (TNP)-labeled P815 mouse mastocytoma cells was measured after a 2½-hour incubation at 37 °C in the presence or absence of neutrophils, rabbit IgG anti-TNP with or without CSF-$\alpha$, human MNC-SN, or MAb WEM-G1. The results are expressed as the percentage of cytotoxicity, determined by the



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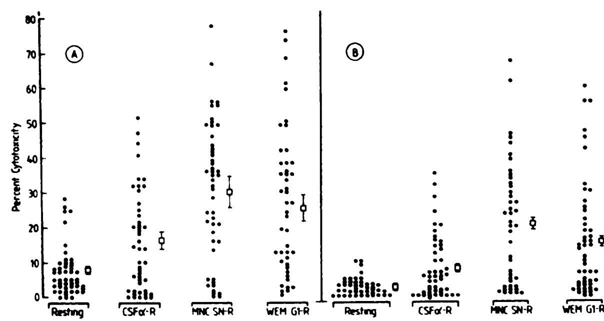

Fig 1. The percentage of cytotoxicity (●) and arithmetic mean (□) ± 1 SEM of the percentage of cytotoxicity by purified neutrophils from 45 to 49 individuals. The cells were either not stimulated in vitro ("resting") or were stimulated by CSF-$\alpha$, MNC-SN, or MAb WEM-G1. In the stimulated case, the cytotoxicity of unstimulated cells is subtracted to give the extent of stimulation. The assays were performed with either a 1/300 (A) or 1/1,000 (B) dilution of rabbit IgG anti-TNP. (A) CSF$\alpha$-R, MNC-SN–R, and WEM-G1–R differ from resting by $P < .001$, and WEM-G1–R and MNC-SN–R are greater than CSF$\alpha$-R at $P < .01$ and $<.001$, respectively. (B) CSF$\alpha$-R, MNC-SN–R, and WEM-G1–R differ from resting by $P < .001$, and WEM-G1–R and MNC-SN–R are greater than CSF$\alpha$-R, at $P < .01$ and $<.001$, respectively.

formula:

$$\frac{\text{Experimental} - \text{background}}{\text{Maximal} - \text{background}} \times 100,$$

where "background" represents the counts released by target cells alone and "maximal" by target cells after lysis by Triton X-100 (Calbiochem-Behring, La Jolla, Calif). Each point is the mean of three replicate determinations. No cytotoxicity was obtained in the absence of rabbit anti-TNP antibody or in the absence of neutrophils. In each experiment, cells from three or four individuals were tested.

## RESULTS

The antibody-dependent cytotoxicity of neutrophils purified from 50 individuals was measured without stimulators ("resting kill") or with the addition of CSF-$\alpha$, MNC-SN (previously shown to contain CSF-like activators),[2] and saturating amounts of the MAb WEM-G1. The results in Fig 1 show that a large degree of heterogeneity existed in all parameters tested using two different dilutions of antitumor target antibody. The slight variations in the purity of neutrophil preparations among different donors could not explain the heterogeneity observed.

*Correlation of "resting" with "stimulated" neutrophil-mediated cytotoxicity.* The correlation between the cytotoxic capacity obtained with "resting" neutrophils and their capacity to become activated (as measured by stimulated – resting) is shown in Table 1. A significant positive correlation was seen between "resting" cytotoxicity and the capacity to become stimulated with CSF-$\alpha$, MNC-SN, or MAb WEM-G1. This suggested that neutrophils were already variably stimulated in vivo and that this stimulation could be more easily demonstrable in "responsive" cells. The heterogeneity in "resting" or "stimulated" cytotoxic capacity was not correlated with blood neutrophil or mononuclear cell counts. Similarly, while there was a variation in the extent to which neutrophils bound WEM-G1, the intensity of the binding did not appear to correlate with their "resting" or "stimulated" cytotoxic capacity in vitro (data not shown). There was no significant difference between normal individuals and patients in these parameters.

*Correlation of the Capacity of Various Stimulating Factors to Activate Neutrophil-Mediated Cytotoxicity.* We found that neutrophils from different individuals responded to a similar degree whether they were stimulated with MAb WEM-G1, CSF-$\alpha$, or MNC-SN (Fig 2). The correlation between the capacity of WEM-G1 and CSF-$\alpha$ to stimulate

Table 1. Correlation of "Resting" and "Stimulated" Neutrophil-Mediated Cytotoxicity

| Variables Correlated $\gamma$ and $P$ Values | | Dilution of Rabbit Anti-TNP IgGc | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1/1,000 | | | | 1/300 | | | |
| | | All | All-C (40) | Normal (15) | Patient (25) | All | All-C (40) | Normal (15) | Patient (25) |
| Resting $v$ CSF$\alpha$-R* | $\gamma$†: | .39† (48) | .44 | .43 | .46 | .47 (45) | .56 | .56 | .56 |
| | $P$‡: | | .004§ | .1 | .02 | | .0002 | .03 | .004 |
| Resting $v$ MNC-SN–R* | $\gamma$: | .45 (48) | .46 | .45 | .48 | .43 (44) | .47 | .37 | .54 |
| | $P$: | | .002 | .09 | .02 | | .002 | .17 | .006 |
| Resting $v$ WEM-G1–R* | $\gamma$: | .39 (49) | .35 | .22 | .46 | .32 (45) | .36 | .35 | .37 |
| | $P$: | | .03 | .4 | .02 | | .02 | .2 | .07 |
| CSF-$\alpha$–R $v$ WEM-G1–R | $\gamma$: | .80 (48) | .82 | .88 | .78 | .76 (46) | .77 | .89 | .68 |
| | $P$: | | <.0001 | <.0001 | <.0001 | | <.0001 | <.0001 | .0002 |
| CSF-$\alpha$–R $v$ MNC-SN–R | $\gamma$: | .81 (47) | .82 | .90 | .78 | .79 (45) | .82 | .87 | .79 |
| | $P$: | | <.0001 | <.0001 | <.0001 | | <.0001 | <.0001 | <.0001 |
| WEM-G1–R $v$ MNC-SN–R | $\gamma$: | .75 (48) | .73 | .73 | .60 | .68 (45) | .69 | .87 | .57 |
| | $P$: | | <.0001 | <.0001 | .002 | | <.0001 | <.0001 | .003 |

Abbreviations: All, all individuals on whom data are available; All-C, all individuals on whom complete data are available; Normal, individuals with no illness (normal laboratory staff members); Patient, individuals from the Royal Melbourne Hospital. Number in each group shown in parentheses. Normal and Patient groups derived from individuals with complete data.

*R denotes cytotoxicity by "resting" or unstimulated neutrophils that were subtracted from the stimulated values of that individual.

†Correlation coefficient determined by regression analysis using the mean of triplicate determinations for each data point.

‡$P$ values as determined by one-tailed $t$ test.

§The correlation coefficient did not differ significantly between the "normal" and "patient" groups.

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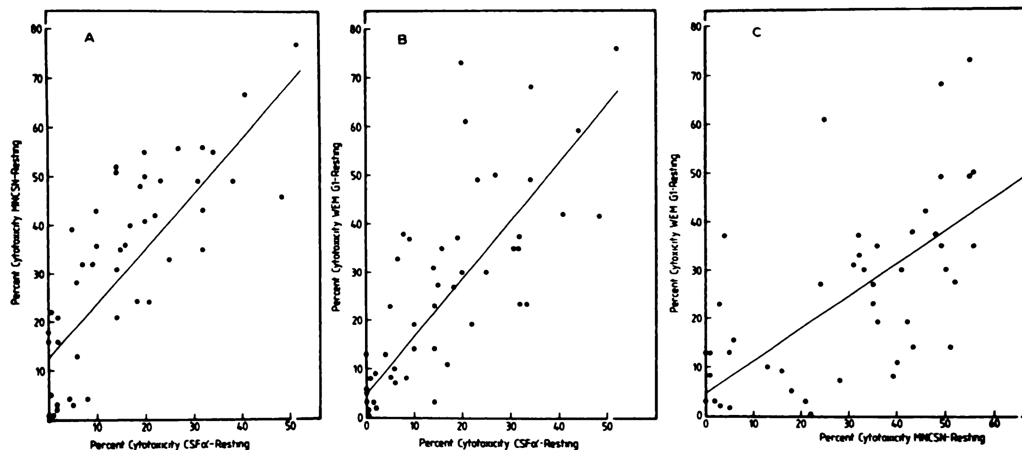


**Fig 2. Correlation between stimulation by MNC-SN and CSF-α (A), WEM-G1 and CSF-α, (B) and WEM-G1 and MNC-SN (C) of neutrophil-mediated cytotoxicity, at rabbit anti-TNP antibody dilution of 1/300. *P* and *r* values are shown in Table 1.**

cytotoxic capacity was calculated by first subtracting "resting" cytotoxicity values, yielding a highly a significant ($P < .0001$) correlation coefficient ($r = .76$). Similarly, a strong and significant ($P < .0001$) correlation was seen when neutrophil stimulation by WEM-G1 was compared with that by MNC-SN ($r = .68$). Stimulation of neutrophils by CSF-α and MNC-SN were also significantly ($P < .0001$) correlated ($r = .79$). These high correlation values were essentially the same whether neutrophils were tested in the presence of antitarget antibody at a dilution of 1/3000 or 1/1000, whether all patients or only those in whom complete data were available were used. Again, there was no difference between the "normal" and "patient" groups in these parameters.

## DISCUSSION

The two major findings in this paper relate to the capacity of human neutrophils to be stimulated by conditioned media containing CSF and by anti-neutrophil MAb. First, a positive correlation was found between the unstimulated cytotoxicity of neutrophils and the degree to which they could be stimulated by our activating agents (Table 1). This finding may be interpreted in terms of an "intrinsic" responsiveness of neutrophils, responsive cells being easily stimulated by factors in vivo (or by the separation procedure) and also responding strongly to in vitro stimulation. The "nonresponsive" neutrophils, by contrast, are poorly stimulated by in vivo or in vitro influences. It is not yet known what determines the "responsiveness" of the cells; however, factors that modulate their surface receptors such as lipopolysaccharide and chemotactic peptides[12] are strong candidates. Interestingly, neither the "patients" in general nor the particular ones with sepsis (data not shown) fall easily into a separate category, and indeed are statistically indistinguishable from the group as a whole. However, before firm conclusions can be drawn in this regard, a larger series will have to be investigated.

The second positive finding is the strong correlation between the stimulating capacity of CSF-containing factors and the MAb WEM-G1. This agains seems to emphasize the importance of neutrophil "intrinsic" responsiveness that dictates a similar degree of activation by signals as diverse as CSF-α and an IgM-Mab. This finding is especially interesting since WEM-G1 is directed against a 110,000-dalton surface glycoprotein and a membrane glycolipid (S. Spitalnik, NIH, personal communication), both appearing mainly on mature cells and neither having the properties of the CSF receptor. Thus, this appears to be the first instance in which a MAb mimics the effect and the effectiveness of a natural regulator without binding to the receptor for the regulator. The lack of correlation in the present series of neutrophil count, monocyte count, and extent of Mab WEM-G1 binding with cytotoxic function cannot be conclusive at this stage and needs further investigation. It was again interesting to observe that these correlations did not differ significantly between "normal" and "patient" groups, suggesting that the capacity to be further stimulated is not abrogated by ongoing disease.

The mechanism whereby resting neutrophils damage tumor cells is not known, although an oxidative burst appears to be an important component.[13,14] In this context, we (A.F.L. and M.A.V., unpublished observations, 1985) and others[15] have found that incubation of neutrophils with conditioned media rich in CSF or recombinant CSF[15] induces production of increased superoxide anion upon stimulation with chemotactic peptides or opsonized zymosan. Thus, oxygen products may be one factor responsible for the enhanced cytotoxicity by neutrophils described in this report.

One clear possibility is raised by our observations: the use of a MAb for the determination of human neutrophil responsiveness and its application to clinical situations. In addition, with new probes available for the measurement of CSF receptor on human neutrophils (N.A. Nicola and M.A. Vadas, manuscript in preparation), the possibility is being tested that the binding of WEM-G1 is related indirectly to the expression of the CSF receptor.

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