Effects of Neuraminidase on the Regulation of Erythropoiesis

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In this article, we present evidence that sialic acid-containing surface components play a role in the regulation of erythropoiesis. A 1-hr exposure of mouse bone marrow cells to high concentrations of neuraminidase reduced erythroid colony formation. Coculture of 10^6 untreated thymocytes with neuraminidase-treated bone marrow cells restored erythroid colony growth. Neuraminidase-treated thymocytes retained their ability to suppress erythroid colony formation by untreated marrow cells, but lost their ability to enhance erythroid colony formation. Continuous exposure to low concentrations of neuraminidase enhanced erythroid bone marrow cell colony growth in response to a suboptimal dose of erythropoietin.

SYNGENEIC THYMOCYTES have been shown to regulate the proliferation of hematopoietic progenitor cells in a number of different experimental systems. We have shown that 10^6 thymocytes cocultured with 5 x 10^6 bone marrow cells in plasma clot stimulate erythroid colony growth, whereas 10^3 thymocytes inhibit colony formation. Although the underlying molecular mechanism is still unclear, it is reasonable to assume that cell surface components participate in the exchange of regulatory signals. One possible approach to the identification of relevant surface structures is to subject the surfaces of progenitor cell and regulatory cell to controlled proteolytic or glycolytic degradation. In this study, we exposed bone marrow cells and thymocytes to a highly purified preparation of *Vibrio cholerae* neuraminidase and determined the effects of the enzyme on thymocyte-mediated modulation of erythropoiesis in vitro.

MATERIALS AND METHODS

**Materials**

Minimal essential medium (MEM) and citrated bovine plasma were obtained from Gibco (Grand Island, NY); a selected batch of fetal bovine serum (heat-inactivated for 1 hr at 56°C) from Sterile Systems (Logan, UT); bovine thrombin (40 U/ml) from Parke-Davis (Morristown, NJ); sheep plasma erythropoietin from Connaught Laboratories (Willowdale, Ontario, Canada); deionized bovine serum albumin from Bio-Rad Laboratories (Richmond, CA); and *Vibrio cholerae* (specific activity: 71.9 U/mg protein, lot 102993), *Arthrobacter ureafaciens* (specific activity: 20 IU/mg protein, lot 102993), and neuramino lactose (from beef colos-
Coculture of $5 \times 10^4$ untreated bone marrow cells with $10^3$ syngeneic thymocytes suppresses erythroid colony formation, whereas coculture with $10^6$ thymocytes causes a marked enhancement of erythropoiesis$^{1,2,5}$ (see Figs. 3 and 5, Table 1). A low number ($10^3$) of thymocytes cocultured with bone marrow cells that had been exposed to neuraminidase for 1 hr did not further suppress erythroid colony growth. Addition of $10^6$ thymocytes reversed the neuraminidase-mediated suppression of erythropoiesis to control levels. In order to determine if residual neuraminidase was responsible for lack of enhancement, bone marrow cells exposed to neuraminidase were treated with neuramine lactose prior to coculture. This also results in reversal of suppression to control levels (Table 1).

In experiments where thymocytes were exposed to neuraminidase for 1 hr and then cocultured with untreated bone marrow cells, thymocytes were unable to enhance erythropoiesis, but continued to suppress erythropoiesis (see Fig. 5). Failure of these thymocytes to enhance erythropoietic growth was also attributable to the enzymatic action of neuraminidase, as the enhancing function was preserved when the neuraminidase treatment was performed in the presence of neuramine lactose (Fig. 4). A mixture of $10^6$ treated and $10^6$ untreated thymocytes cocultured with untreated bone marrow cells enhanced erythroid colony formation (Fig. 5). Continuous exposure of cultured bone marrow cells to neuraminidase also affected erythroid colony growth. Supplementation of culture medium with a low concentration (0.0025–0.005 IU/ml) of neuraminidase enhanced the response of CFU-E to suboptimal concentrations of erythropoietin. However, no erythroid colonies were formed in the absence of erythropoietin. The high (0.1 IU/ml) concentration of neuraminidase was inhibitory (Table 2).

**DISCUSSION**

Effects of neuraminidase on spleen colony-forming cells (CFU-S) and erythroid progenitor cells (CFU-E, BFU-E) have been reported.$^{21-28}$ Some authors have reported on inhibition of erythroid colony formation,$^{21,26}$ and others noted enhancement of erythroid colony formation or hemoglobin synthesis.$^{27,30}$ These discrepancies may, however, be more apparent than real. The contradictory results were obtained with different assay systems, different enzyme preparations, and different experimental protocols. The most significant results of this communication are: (1) the same preparation of highly purified neuraminidase could both enhance and suppress erythroid colony formation, depending on the concentration of the
Table 1. Effect of Thymocytes Cocultured With Neuraminidase-Treated Bone Marrow Cells (With or Without Neuramine Lactose Incubation)

<table>
<thead>
<tr>
<th>Thymocytes Added</th>
<th>GFH†</th>
<th>VCN‡</th>
<th>GFH + NL§</th>
<th>VCN + NL‖</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55.0 ± 5.0</td>
<td>16.0 ± 1.8</td>
<td>42.8 ± 4.8</td>
<td>9.5 ± 1.8</td>
</tr>
<tr>
<td>10³</td>
<td>20.5 ± 0.5</td>
<td>26.0 ± 3.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10⁴</td>
<td>39.6 ± 5.5</td>
<td>33.1 ± 1.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10⁵</td>
<td>36.6 ± 4.0</td>
<td>38.8 ± 2.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10⁶</td>
<td>116.3 ± 14.0</td>
<td>55.5 ± 2.0</td>
<td>184.6 ± 21.1</td>
<td>38.0 ± 0.8</td>
</tr>
</tbody>
</table>

*This value represents the mean ± SEM for 4 plasma clots.
†Glucose-free Hanks' buffer.
‡Vibrio cholerae neuraminidase, 0.1 IU/ml.
§Glucose-free Hanks' buffer followed by a second incubation with neuramine lactose.
‖Vibrio cholerae neuraminidase followed by a second incubation with neuramine lactose.

effect of neuraminidase on erythroblast colony growth was only observed when small amounts of neuraminidase were added directly to the culture media. This effect was probably attributable to the desialylation of erythropoietin and high net negative surface charge that probably reflects a high degree of sialylation of cell surface molecules (Fig. 3–5). By contrast, suppressing cells appear to be a less mature population of thymocytes characterized by a larger cell volume, a low surface density of theta antigen, fewer sialic acid residues on the cell surface, sensitivity to radiation, 4-hydroperoxycyclophosphamide, and difluoromethylornithine243 (Fig. 3 and 5, Table 1), and to basic polyamino acids.7 Although these populations have been characterized, they remain to be isolated in pure form. Until such isolation is accomplished, absolute functional capacity remains an open question.

The inhibitory effects of a short-term exposure of bone marrow cells to high concentrations of neuraminidase were offset by the addition of 10⁶ thymocytes. As thymocytes alone form no erythroid colonies,1 the
neuraminidase treatment could have affected a regulatory cell population of the bone marrow. However, a simultaneous effect on erythroid progenitor cells could not be excluded, because the addition of thymocytes restored colony growth only to control levels (Table 1). One possibility was that neuraminidase bound to marrow cells, was neutralized by the addition of (10⁶) thymocytes, and prevented enhancement of colony formation. We believe this to be extremely unlikely because: (1) a further incubation of neuraminidase-treated bone marrow cells with neuramine lactose prior to culture had no effect on the suppression of erythroid colony growth (Table 1), and (2) the addition of 10⁶ thymocytes to treated marrow cells that were exposed to neuramine lactose in order to neutralize bound neuraminidase, either in preculture or continuously, also resulted in restoration of erythroid colony growth but not enhancement by thymic regulatory cells. Thus, helper activity is unlikely to be due to neutralization of residual cell-bound neuraminidase.

We do not know whether neuraminidase treatment is selectively cytotoxic for subpopulations of regulatory cells or progenitor cells or whether it merely alters their function. If it alters their function, at least two different mechanisms have to be considered to explain the inhibitory effects of neuraminidase treatment on regulatory T cells and/or erythroid progenitor cells: (1) high affinity binding of the enzyme to the cell surface, which might result in steric interference with surface structures essential for normal function, and (2) consequences of the catalytic action of the enzyme at the cell surface or inside the cell after possible endocytosis of the adsorbed enzyme. Neuraminidase-treated thymocytes (10⁶) appear to function as suppressor cells (Fig. 5). The suppressor activity may be due to newly exposed cell surface molecules after removal of sialic acid (i.e., penultimate galactose residues). Such interactions may lead to suppressor activity and result in a reduction in CFU-E formation. In preliminary studies, we found that a further modification of the cell surface by altering galactose residues (utilizing galactose oxidase) eliminated the suppressor function of thymocytes, as galactosyl residues might be associated with suppressor activity. Thus, thymocyte regulation may be altered by cell surface modification. At present, we are examining the role of newly exposed molecules in the suppressor activity of thymocytes.

These data suggest that the cell surface of thymic regulatory cells is involved in the control of erythroid progenitor cell growth. Presumably, this process is mediated through specific cell surface molecules or molecular arrays (i.e., containing sialic acid residues). Further identification of specific membrane constituents that mediate intercellular communication are of fundamental importance for the control of erythroid progenitor cell growth.

**REFERENCES**


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