Correlation of Messenger RNA Levels With Protein Defects in Specific Granule Deficiency

By Jennifer J. Johnston, Laurence A. Boxer, and Nancy Berliner

Neutrophil specific granule deficiency (SGD) is a rare congenital disorder of unknown cause associated with an impaired inflammatory response and an absence of neutrophil secondary granules. Reduced levels of several neutrophil proteins have led to the suggestion that the defect may lie at the level of transcription, a hypothesis that is supported by abnormally low levels of lactoferrin message in the bone marrow of two SGD patients. We have examined the level of seven granule protein RNAs in one SGD patient and have compared them with reported protein levels. We have found the RNA levels for all of these genes to be reduced in proportion to the decreased levels of their respective proteins. These data further support the hypothesis that the reduced protein levels reflect a defect in transcriptional control.

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Granulocyte differentiation progresses through several well-defined stages marked by characteristic morphologic changes. These changes include progressive nuclear segmentation as well as the acquisition of stage-specific granules. As the cell progresses to the myelocyte stage, secondary (specific) granules are acquired and the cell becomes committed to terminal neutrophil differentiation.1,2 Secondary granules and the expression of secondary granule content protein genes, therefore, provide good markers for commitment to terminal granulocytic maturation.

Specific granule deficiency (SGD) is a rare congenital disorder in which patients present with recurrent infections attributable to functional neutrophil abnormalities.3 Microscopic examination of patient neutrophils shows morphologic abnormalities of nuclear lobulation and membrane configuration, as well as a near absence of secondary granules.4,5 The cells display abnormal cell motility, defective bactericidal activity,6,7 and a heightened state of activation.8 Biochemical studies have shown several neutrophil proteins to be present at lower than expected levels, including but not limited to the full range of secondary granule content proteins.9 Protein levels for both transcobalamin I and lactoferrin, two of the secondary granule content proteins, have been shown to be reduced in patient plasma but normal in saliva from at least one individual. This finding suggests that the defect is restricted to neutrophils.7

Specific granule deficiency is further support the hypothesis that the reduced protein levels reflect a defect in transcriptional control.

We describe here our analysis of BM RNA from one of these patients9 looking at the levels of steady-state RNA encoding a wide range of affected proteins. To determine if the RNA defect is specific to lactoferrin, we have examined the BM RNA levels for transcobalamin I, neutrophil collagenase, neutrophil gelatinase, and defensins. All of these RNAs are shown to be produced at a reduced level when compared with normal BM. We have also examined the RNA levels of CD18, an adhesion protein that is a component of both the plasma membrane and the secondary granule membrane. Flow cytometric analysis of CD18 has shown its presence on the SGD neutrophil membrane, but it fails to undergo the increase in membrane expression normally seen upon neutrophil activation.10 The RNA level for CD18 is shown to be unaffected.

Materials and Methods

Patient Information. Normal BM (NBM) was obtained from anonymous discarded samples retrieved after clinical procedures were performed. The SGD patient sample was obtained for this study after informed consent. The patient has been described previously.8 Briefly, MF is a 35-year-old man clinically free of any infection at the time of study. The patient’s BM aspirate showed normal cellularity, a myeloid:erythroid (M:E) ratio of 1.5:1, and the following cellular differential: 19% neutrophils, 1% band forms, 6% metamyelocytes, 26% myelocytes, 5% promyelocytes, 37% red blood cell (RBC) precursors, 6% lymphocytes, 1% eosinophilic metamyelocytes, and 1% monocytes. This is comparable to previous marrow from this patient.

RNA isolation. White blood cells (WBCs) were separated from patient and normal BM using sedimentation of the RBCs followed by RBC lysis in isotonic solution. After adding 0.15 mL acid citrate dextrose per milliliter of aspirate as an anticoagulant, the aspirate was mixed with an equal volume of 3% dextran T-500 in 0.9% NaCl, and incubated at room temperature for 45 minutes. The buffy coat was centrifuged at 200g for 10 minutes at 4°C and the pellet was resuspended in ice-cold distilled H2O for 30 seconds and then brought to 0.9% NaCl. After repeat centrifugation, the pellet

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Table 1. Representative Protein Levels From an SGD and a Normal Individual, With Comparison of RNA and Protein Ratios

<table>
<thead>
<tr>
<th>Protein &amp; Amount</th>
<th>SGD</th>
<th>Normal</th>
<th>SGD:Normal Protein Ratio</th>
<th>Reference</th>
<th>SGD:Normal RNA Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme (μg/10⁶ PMN)</td>
<td>86.8 ± 14.5</td>
<td>113.7 ± 30.6</td>
<td>ND</td>
<td>8</td>
<td>ND</td>
</tr>
<tr>
<td>MPO (nmol/L/10⁶ PMN)</td>
<td>900 ± 200</td>
<td>1160 ± 20</td>
<td>128%</td>
<td>8</td>
<td>100% (defined)</td>
</tr>
<tr>
<td>Lactoferrin (μg/10⁶ PMN)</td>
<td>70 ± 15</td>
<td>2.4 ± 0.1</td>
<td>3.4%</td>
<td>8</td>
<td>0.9%</td>
</tr>
<tr>
<td>B₂₃ transport protein (pg/10⁶ PMN)</td>
<td>4,320</td>
<td>350</td>
<td>8.1%</td>
<td>8</td>
<td>12.3%</td>
</tr>
<tr>
<td>Alkaline phosphatase*</td>
<td>Undetectable</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
<td>ND</td>
</tr>
<tr>
<td>Gelatinase†</td>
<td>1,213 ± 32</td>
<td>7 ± 4.6</td>
<td>0.6%</td>
<td>9</td>
<td>3.7%</td>
</tr>
<tr>
<td>Defensins§</td>
<td>100%</td>
<td>10%</td>
<td>10%</td>
<td>10</td>
<td>12.5%</td>
</tr>
<tr>
<td>CD18§</td>
<td>19.57</td>
<td>9.68</td>
<td>49%</td>
<td>9</td>
<td>149%</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.
*By histochemical staining.
†10⁶ PMN released in response to 10⁻⁶ mol/L A23187.
§Based on immunoreactivity with control set to 100%.
\[SCREEN\] Variability between genes, but corresponds to the levels seen for the individual proteins (Table 1). Results from densitometry are included in Table 1 and are reported as ratios of SGD to normal RNA, normalized to MPO expression, which was defined as 100%. Protein ratios have also been computed for comparison. MPO, known to be unaffected at

RESULTS

Total RNA was isolated from the BM leukocytes from an SGD patient and a control individual, as well as from the peripheral blood leukocytes from an individual with chronic myelogenous leukemia (CML). Figure 1 shows a Northern blot of these RNA samples probed for neutrophil collagenase, neutrophil gelatinase, lactoferrin, transcobalamin I, defensins, CD18, and MPO transcripts. Lane 1 contains SGD patient BM RNA, lane 2 contains NBM RNA, and lane 3 contains CML peripheral blood RNA. Neutrophil collagenase, neutrophil gelatinase, lactoferrin, transcobalamin I, and defensins are all detected at reduced levels in lane 1 as compared with lane 2. The level of reduction varies between genes, but corresponds to the levels seen for the individual proteins (Table 1). Results from densitometry are included in Table 1 and are reported as ratios of SGD to normal RNA, normalized to MPO expression, which was defined as 100%. Protein ratios have also been computed for comparison. MPO, known to be unaffected at
the protein level in SGD, is not reduced at the RNA level in the SGD leukocytes, CD18 is actually increased at the RNA level in SGD as compared with the control. All transcripts are detected in the CML RNA.

DISCUSSION

Specific granule deficiency is a syndrome characterized by numerous defects in patient neutrophils. Along with an apparent absence of secondary granules, there is a reduction in the level of numerous proteins including, but not limited to, secondary granule content proteins. Lactoferrin and TCI are produced at normal levels in other tissues, suggesting that the defect is restricted to neutrophils. Furthermore, only a subset of neutrophil proteins are affected, which suggests that the defect is not general.

Defects in both gene expression and protein processing have been suggested as mechanisms that might account for the abnormal reduction in protein levels in SGD neutrophils. Abnormal intracellular transport to the granule or defective movement of proteins into abnormal granules could lead to rapid degradation of free granule content proteins within the cytoplasm. However, normal production and packaging of myeloperoxidase into primary granules, suggests that this is not the case in SGD. Furthermore, the absence of the primary granule defensins, despite otherwise apparently normal primary granules, further suggests that the defect is a primary defect of protein production.

Our data, in conjunction with the previous experiments by Lomax et al looking at lactoferrin RNA levels, strongly suggest that the defect in SGD has an impact on steady-state levels of messenger RNA. We have studied the RNA levels for several genes encoding proteins known to be affected in SGD to determine if their RNA levels also correspond with their reduced protein levels. We have shown that the steady-state RNA levels for neutrophil collagenase, neutrophil gelatinase, TCI, and defensins are all reduced in the BM from one SGD patient. CD18, a protein present on the membrane of the secondary granule, has been shown to be present at normal levels on SGD neutrophils. The RNA level is increased in the SGD sample as compared with normal, with a ratio of 149%. Because increased surface expression of CD18 in stimulated neutrophils results from fusion of secondary granules with the plasma membrane, the failure of SGD neutrophils to exhibit increased CD18 after f-Met-Leu-Phe (FMLP) stimulation presumably reflects the absence of secondary granules in those cells.

These data suggest that the most likely explanation for the observed protein defects is a primary failure of RNA accumulation. Such a lesion could be the result of either a transcriptional defect or a lesion affecting RNA stability. Parallel changes in RNA stability of multiple genes would be expected to be dependent on shared sequences determining RNA metabolism. Analysis of the cDNA sequences of these proteins shows no such homologous regions predicting common RNA degradation pathways. Consequently, we feel that this is an unlikely explanation for the observed protein deficit. We postulate that SGD reflects a primary defect in gene expression at the level of transcription, possibly in a shared transcription factor common to the affected subset of proteins. Definitive confirmation of this would be best provided by nuclear run-on assays. However, BM cells are unavailable for such studies.

SGD is the first disease syndrome in which a seemingly unrelated group of proteins have been found to be affected at the RNA level. As such, it presents a unique opportunity to study the coordinate expression of a group of diverse genes related by the time of their appearance during myeloid differentiation. This has led us to begin studying the promoters of these genes to find regulatory regions shared between them in hopes of identifying a common transcription factor affected in SGD.

REFERENCES


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