Further Evaluation of Luminol-Enhanced Luminescence in the Diagnosis of Disorders of Leukocyte Oxidative Metabolism: Role of Myeloperoxidase

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Chemiluminescence can be used to identify defects in the oxidative metabolism of granulocytes. This procedure has recently been adopted for use with microliter quantities of whole blood, appropriate for prenatal or neonatal study. Although the contribution of myeloperoxidase to the chemiluminescence assay has been noted, the possible diagnostic confusion between chronic granulomatous disease of childhood (which is rare and severe) and myeloperoxidase deficiency (which is common and of little clinical consequence) has not been stressed. We report a father and his infant daughter whose cells emitted no light in the luminol-enhanced luminescence assay; both patients are totally peroxidase deficient. These results emphasize the hereditary nature of myeloperoxidase deficiency, and the possibility for erroneous diagnosis of chronic granulomatous disease of childhood based on the luminol-enhanced luminescence test.

Additional Keyphrases: heritable disorders • chronic granulomatous disease of childhood

Polymorphonuclear leukocytes (PMN) are critical in defense against bacterial and fungal pathogens (1). The recent years have become clear that the ability of PMNs to reduce molecular oxygen to hydrogen peroxide (H2O2) is of considerable importance in the microbialic process (1–4). Furthermore, the interaction of H2O2, myeloperoxidase, and a halide (chloride, in the physiologic setting) dramatically enhances oxidative killing (1, 4).

Both chronic granulomatous disease of childhood (CGD) and myeloperoxidase deficiency are hereditary disorders (1, 3, 5–8). Granulocytes in CGD do not form superoxide (O2) or H2O2, and affected patients are susceptible to frequent, severe infections (2, 3, 5). Myeloperoxidase deficiency, on the other hand, appears to be a common disorder from which most patients suffer no adverse consequences (6–8). Although various diagnostic tests are available for CGD, the chemiluminescence test has become increasingly popular because of ease of performance and its enhanced sensitivity in the presence of luminol (9, 10). Recently this assay has been adopted to a microtechnique that can be used on whole blood (11–13), making it particularly appropriate for use in infants and neonates. The biological basis for light emission in this assay is unknown, but it appears to depend on the formation of one or more oxygen reduction products (e.g., O2; H2O2, or both) (1). Although several investigators have noted a relationship between PMN chemiluminescence and myeloperoxidase concentration (10, 12, 14–16), the potential confusion between the diagnosis of CGD and myeloperoxidase deficiency has not been adequately emphasized, in part because of the disparate results in the degree of abnormality observed by different investigators (10, 12, 14, 15). In this study we examined the luminol-dependent luminescence of a family in which the PMNs of a father and infant daughter were totally peroxidase deficient. Our results suggest that peroxidase deficiency per se could lead to an erroneous diagnosis of CGD when the luminol-dependent luminescence assay is used. Furthermore, the presence of peroxidase deficiency in the neonate examined further emphasizes the hereditary nature of this disorder.

Materials and Methods

Family study: The proband, G.P., was a 31-year-old white man in excellent health with no history of recurrent infections or febrile episodes. During routine blood donation for a research protocol his granulocytes were discovered to be markedly deficient in light emission in a luminol-dependent assay, and the possibility of CGD was entertained; however, his clinical history did not support this diagnosis. A.P., the six-week-old daughter of the proband, was the product of an uncomplicated full-term pregnancy. During the fourth week of life she developed rhinorrhea, which resolved without therapy. B.P., the 28-year-old mother of A.P., had no medical problems. None of the subjects were taking medication.

Subsequent to obtaining informed consent, in accordance with the guidelines of the Human Investigation Committee of University of North Carolina, we obtained blood from G.P. and B.P. by venipuncture, and from A.P. by heel stick. The blood was immediately heparinized (Panheparin; Abbott Diagnostics, N. Chicago, IL 60064) and thoroughly mixed by gentle rocking before preparation of wedge-pulled smears. Total and differential leukocyte counts were determined with an automated cytochemical analyzer (Hemalog D; Technicon Instrument Co., Tarrytown, NY 10591) (7, 8), and manual differential leukocyte counts were determined from Wright–Giemsa stained smears.

Peroxidase determination: In related studies we used various methods to show that the cells of the proband (G.P.) were totally deficient in myeloperoxidase (14). In the present study we examined the cells for peroxidase by using the Hemalog D, and by manual examination. In both procedures 4-chloro-1-naphthol was used as the cytochemical stain (7, 8).

Luminol-dependent chemiluminescence: Light emission by phagocytic cells in whole blood was measured in a luminol-enhanced system as previously described (11). Briefly, luminol (Sigma Chemical Co., St. Louis, MO 63178) was frozen at 10 g/L in dimethylsulfoxide and diluted 5000-fold with Dulbecco's phosphate-buffered saline to a final concentration of 10 μmol/L. Chemiluminescence was measured with a LS 100 C liquid scintillation counter (Beckman Instruments, Fullerton, CA 92634) in the out-of-coincidence

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4 Nonstandard abbreviations: PMN, polymorphonuclear leukocytes; CGD, chronic granulomatous disease; PMA, phorbol myristate acetate.

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mode with a $^{14}$C–$^{3}$H window and a gain of 2.5. Reaction mixtures were 100 $\mu$L of whole blood and 0.20 mL of luminol and brought to a final volume of 2 mL with phosphate-buffered saline in 4-mL polyethylene mini-vials. Cells were stimulated with phorbol myristate acetate (PMA) at a final concentration of 1.0 mg/L.

Results

As shown in Table 1, examination of the peripheral smear of all three family members revealed a normal leukocyte differential count. Myeloperoxidase activity in patient G.P. was unmeasurable by automated cytochemical analysis, o-dianisidine spectrophotometric determination, or alanine decarboxylation, as previously reported (14). None of the neutrophils in smears obtained from patients G.P. or A.P. were peroxidase positive. In contrast, 100% of B.P.'s neutrophils displayed peroxidase activity.

Luminol-dependent chemiluminescence was determined in whole blood as previously described (11). Both resting and PMA-stimulated light emission were measured. Whereas cells from B.P. demonstrated activity equivalent to those of a normal donor, no light emission was detected in blood from A.P. or G.P. (Figure 1).

Discussion

Chemiluminescence has been used extensively to identify patients with CGD and carriers of the disease (10–12, 14, 17), whose granulocytes invariably demonstrate absent or decreased light emission. The increased sensitivity of the chemiluminescence assay in the presence of the cyclic hydrazide luminol has been emphasized (9–13, 17), especially relative to nitro blue tetrazolium dye reduction (11, 17), an assay of $O_{2}$-generation commonly used in the diagnosis of CGD. The latter is cumbersome, only qualitative, and subject to interpretation error; results of the stimulated dye-reduction test, however, are normal in patients with myeloperoxidase deficiency (5).

The chemical basis of granulocyte chemiluminescence has been vigorously addressed. The available data suggest that luminol-dependent luminescence results from the formation of hypochlorous acid through the interaction of $H_{2}O_{2}$, granulocyte myeloperoxidase, and chloride (10, 14–16). Luminol-dependent luminescence is decreased in cells treated with azide (which inhibits myeloperoxidase), and in cells totally deficient in peroxidase (10, 14–16). This is in contrast to luminescence in the absence of luminol, where at least part of the light emitted appears to be superoxide dependent, and another part peroxidase dependent (14–16); peroxidase-deficient neutrophils persist to generate a detectable luminescence when luminol is not included in the assay (14).

In this study luminol-dependent luminescence could not be stimulated in whole blood obtained from a father and his infant daughter, both of whom had granulocytes totally deficient in peroxidase activity. Myeloperoxidase deficiency may be inherited (6–8) or acquired in association with other hematologic abnormalities (18–21). The peroxidase deficiency in patient A.P., documented in her third and sixth week of life, emphasizes the hereditary nature of the disorder and shows that the deficiency is manifested at or soon after birth. However, we have formed no firm conclusion regarding the mode of inheritance of this disorder (6–8).

These results highlight the limitations of the luminol-dependent luminescence assay in the diagnosis of CGD. This disorder is rare, with only approximately 250 cases reported (2), and is associated with repeated severe infections (2, 3, 5). Hereditary peroxidase deficiency occurs as frequently as 1 per 2100 and appears to be of much less clinical significance (8). Our results show that a patient with total peroxidase deficiency could be labeled with the diagnosis of CGD if luminol-dependent luminescence is used for diagnosis, a conclusion consistent with other studies involving peroxidase-deficient cells (10). Furthermore, patients with partial peroxidase deficiency may manifest a less severe, but detectable, abnormality in light emission (unpublished observations). When luminol-dependent luminescence is used for clinical evaluation of PMN function or other experimental studies, the peroxidase concentration in the cells used should be carefully considered.

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