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*J Immunol* 2004; 172:6373-6381; doi: 10.4049/jimmunol.172.10.6373

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6-Phosphogluconate Dehydrogenase and Glucose-6-Phosphate Dehydrogenase Form a Supramolecular Complex in Human Neutrophils That Undergoes Retrograde Trafficking during Pregnancy

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Neutrophils from pregnant women display reduced neutrophil-mediated effector functions, such as reactive oxygen metabolite (ROM) release. Because the NADPH oxidase and NO synthase produce ROMs and NO, the availability of their substrate NADPH is a potential regulatory factor. NADPH is produced by glucose-6-phosphate dehydrogenase (G-6-PDase) and 6-phosphogluconate dehydrogenase (6-PGDase), which are the first two steps of the hexose monophosphate shunt (HMS). Using immunofluorescence microscopy, we show that 6-PGDase, like G-6-PDase, undergoes retrograde transport to the microtubule-organizing centers in neutrophils from pregnant women. In contrast, 6-PGDase is found in an anterograde distribution in cells from nonpregnant women. However, lactate dehydrogenase distribution is unaffected by pregnancy. Cytochemical studies demonstrated that the distribution of 6-PGDase enzymatic activity is coincident with 6-PGDase Ag. The accumulation of 6-PGDase at the microtubule-organizing centers could be blocked by colchicine, suggesting that microtubules are important in this enzyme's intracellular distribution. In situ kinetic studies reveal that the rates of 6-glucuronate turnover are indistinguishable in samples from nonpregnant and pregnant women, suggesting that the enzyme is functionally intact. Resonance energy transfer experiments showed that 6-PGDase and G-6-PDase are in close physical proximity within cells, suggesting the presence of supramolecular enzyme complexes. We suggest that the retrograde trafficking of HMS enzyme complexes during pregnancy influences the dynamics of NADPH production by separating HMS enzymes from glucose-6-phosphate generation at the plasma membrane and, in parallel, reducing ROM and NO production in comparison with fully activated neutrophils from nonpregnant women. The Journal of Immunology, 2004, 172: 6373–6381.
The superoxide and NO produced yield additional downstream ROMs and RNIs. ROM and RNI production require electrons in the form of NADPH, as illustrated in Equations 1 and 2. NADPH production, in turn, requires the hexose monophosphate shunt (HMS) and glucose. For example, neutrophils do not produce superoxide anions in the absence of glucose (17–19). To drive the HMS, glucose transport, a rate-controlling step in metabolism (20), is accelerated by neutrophil activation (21). One process that increases glucose transport is hexokinase translocation to the plasma membrane (22), where it catalyzes the formation of glucose-6-phosphate (G-6-P). G-6-P is metabolized by the HMS, a cell’s primary NADPH source, and by glycolysis. The first step of the HMS is mediated by glucose-6-phosphate dehydrogenase (G-6-PDase), which converts G-6-P into 6-phosphogluconolactone (6-PG) with the release of NADPH. 6-Phosphogluconate dehydrogenase (6-PGDase) converts 6-PG into ribose 5-phosphate and NADPH. We have recently discovered that the intracellular trafficking of G-6-PDase regulates the HMS and, in turn, ROM production (23). In nonpregnant individuals, G-6-PDase is located at the cell periphery where G-6-P is produced by hexokinase and is readily available. However, in pregnant women, G-6-PDase undergoes retrograde transport on microtubules to a cell’s microtubule-organizing center (MTOC) (23). In this location, G-6-P is less available to G-6-PDase, because it is metabolized by glycolytic enzymes at the cell periphery. In the present study, we extend these previous observations by showing that 6-PGDase undergoes similar retrograde trafficking during pregnancy. Moreover, 6-PGDase and G-6-PDase appear to form a complex within cells, which may account for their parallel trafficking, and the accompanying reduction in ROM and NO release.

Materials and Methods

Patients
Peripheral blood samples were obtained from nonpregnant women and pregnant women after written informed consent was provided. The collection of specimens for the study of inflammatory mechanisms was approved by the Institutional Review Board. The nonpregnant group consisted of women in the secretory phase of the menstrual cycle who were not taking oral contraceptives and who had no history of acute or chronic inflammatory conditions (such as asthma or recent infections). Women with normal pregnancies had no medical or obstetric complications, and their pregnancies ranged in gestational age from 20 wk to term. Eligible patients were approached at the Detroit Medical Center/Wayne State University (Detroit, MI).

Cell preparation
Neutrophils were isolated from blood samples using Ficoll-Hypaque (Sigma-Aldrich, St. Louis, MO) density gradient centrifugation (23). Neutrophil viability was >95% as assessed by trypan blue exclusion. Cells were suspended in HBSS (Life Technologies, Grand Island, NY).

Reagents and Abs
Colchicine, LPS (serotype 026:B6), melatonin, and FMLP were obtained from Sigma-Aldrich, FITC and tetramethylrhodamine isothiocyanate (TRITC) were obtained from Molecular Probes (Eugene, OR). Rabbit anti-G-6-PDase and goat anti-lactate dehydrogenase (LDH) polyclonal Abs were obtained from Chemicon International (Temecula, CA). Anti-γ-tubulin was obtained from R&D Systems (Minneapolis, MN). Anti-6-PGDase (S4D5) was prepared as previously described (24). FITC- or TRITC-conjugated Abs were prepared as described (25).

6-PGDase cytometry
6-PGDase activity was studied using cytometric methods (26, 27). Briefly, the 6-PGDase incubation medium consisted of phosphate buffer (pH 7.4), 32% (w/v) polyvinyl alcohol, 2 mM 6-phosphogluconate (VWR, Batavia, IL), 0.4 mM NADP+ (Calbiochem, San Diego, CA), 2.5 mM MgCl2, 2.5 mM NaH2PO4, 0.16 mM L-methionine methosulfate (Sigma-Aldrich), and 2.5 mM Nitro BT (VWR). G-6-PDase and LDH were stained by a similar procedure (26, 27). Cells were washed, fixed with 2% paraformaldehyde, and then incubated for 30 min. Samples were washed to stop the reaction. The samples were transilluminated using a 590 long-pass optical filter (Omega Optical, Brattleboro, VT) to enhance the contrast of the reaction product relative to background.

6-PGDase activity was also evaluated using quantitative microphotometry. To evaluate 6-PGDase kinetics, samples were incubated with reagent while being viewed microscopically. In these experiments the iris was reduced in size to minimize the illuminated region, thus reducing stray light.

Immunofluorescence staining
Neutrophils were placed on glass coverslips, incubated with reagents as described below, and then fixed with Naftalan’s protocol (22). Briefly, cells were fixed with 2% paraformaldehyde, permeabilized with 1% Brij-58, and fixed with 2% paraformaldehyde at room temperature for 20 min. Cells were washed with HBSS, labeled with 1 μg of FITC and/or TRITC-conjugated Abs at 4°C for 30 min, and then washed again with HBSS at room temperature.

Fluorescence microscopy
Cells were observed using an Axiovert fluorescence microscope (Carl Zeiss, New York, NY) with mercury illumination interfaced to a computer using Scion image-processing software (28). A narrow bandpass discriminating filter set (Omega Optical) was used with excitation at 485/22 nm and emission at 530/30 nm for FITC, and an excitation of 540/20 nm and emission at 590/30 nm for TRITC. Long-pass dicroic mirrors of 510 and 561 nm were used for FITC and TRITC, respectively. For resonance energy transfer (RET) imaging, 485/22- and 590/30-nm optical filters were used for excitation and emission, respectively, in conjunction with a 510-nm dicroic mirror. The fluorescence images were collected with an intensified charge-coupled device camera (Princeton Instruments, Princeton, NJ).

NAD(P)H oscillations
NAD(P)H autofluorescence oscillations were detected as previously described (23). Briefly, a 365WB50 excitation filter, a 400-nm long-pass dicroic mirror, and a 450AF58 emission filter were used. A cooled high-sensitivity photomultiplier tube in a D104 detection system (Photon Technology, Lawrenceville, NJ) attached to a Zeiss microscope was used. Data were analyzed using Felix software (Photon Technology).

Single-cell emission spectrophotometry
Energy transfer was also examined by means of microscope spectrophotometer apparatus (29, 30). Fluorescence emission spectra were collected from single cells by a Peltier-cooled IMAX camera with a liquid nitrogen-cooled intensifier (Princeton Instruments) attached to a modified Zeiss Axiovert fluorescence microscope. Microspectrophotometry used a 485/22-nm narrow bandpass discriminating filter for excitation, a 510-nm long-pass dicroic mirror, and a 520-nm long-pass emission filter. WinSpec software (Princeton Instruments) was used to analyze spectrophotometric data.

Detection of ROM and NO production
Pericellular release of ROMs from single cells was monitored as described (31). Briefly, adherent neutrophils were surrounded in 2% gelatin containing 100 ng/ml dihydrotetramethylrosamine (Molecular Probes). ROMs, especially H2O2, released by the cells entered the gelatin matrix, where they oxidized dihydrotetramethylrosamine to tetramethylrosamine, which was detected by fluorescence microscopy. NO production was monitored using diaminofluorescin-2 as previously described (32).

Results
6-PGDase is found at the MTOC in neutrophils from normal pregnant women, but not nonpregnant women
We have recently reported that G-6-PDase undergoes retrograde trafficking in neutrophils from pregnant women, whereas anterograde trafficking is found in cells from nonpregnant women (23). Because 6-PGDase is another key NADPH-producing component of the HMS, we hypothesized that 6-PGDase undergoes specific translocation to the MTOC of neutrophils from pregnant women, thus leading to a complete spatial polarization of glycolysis and the NADPH-producing steps of the HMS. To test this hypothesis, we examined the intracellular distribution of 6-PGDase in neutrophils.
from nonpregnant and pregnant women with and without in vitro stimulation with LPS using immunofluorescence microscopy. As illustrated in Fig. 1, the anti-6-PGDase label is found primarily at the periphery of neutrophils from nonpregnant women (Fig. 1a). Untreated neutrophils, whether spherical or polarized in shape, show this intracellular distribution of 6-PGDase (data not shown). As HMs activation may alter the trafficking of its constituent enzymes, we evaluated the effect of LPS, a reagent known to stimulate cells, on the intracellular distribution of 6-PGDase. Indistinguishable results were obtained when neutrophils were exposed to LPS (50 ng/ml) for 20 min (Fig. 1, a and b). We next examined the intracellular location of 6-PGDase in cells from pregnant women. In contrast to neutrophils from nonpregnant women, 6-PGDase is found in the vicinity of the MTOC in cells from pregnant women (Fig. 1, g and h) in the presence and absence of LPS exposure. As a positive control, similar changes were noted for G-6-Pase (Fig. 1, c, d, i, and j). As a negative control, the intracellular distribution of LDH in neutrophils from pregnant and nonpregnant women in the presence of LPS stimulation was evaluated (Fig. 1, e, f, k, and l). The LDH distribution was not effected by pregnancy or LPS stimulation. Similar negative controls with other metabolic enzymes including phosphofructokinase (PFK) and pyruvate kinase have been reported previously (23). Thus, 6-PGDase undergoes differential trafficking in cells from pregnant and nonpregnant women.

** FIGURE 1.** Immunofluorescence microscopy of 6-PGDase, G-6-Pase, and LDH in neutrophils. Representative cells from nonpregnant women (top row; a-f) and pregnant women (bottom row; g-l) are shown. Neutrophils were stained with and without prior incubation with LPS (50 ng/ml, 20 min). Cells were fixed as described (22), and then stained with polyclonal Abs directed against 6-PGDase (a, b, g, and h), G-6-Pase (c, d, i, and j), and LDH (e, f, k, and l). Anti-6-PGDase is peripherally located in cells from nonpregnant women (a and b), but was found near the center of cells from pregnant women (g and h). Thus, 6-PGDase undergoes differential trafficking in cells from nonpregnant and pregnant women. As a positive control, similar trafficking of G-6-Pase was observed (c, d, i, and j). As a negative control, LDH did not undergo intracellular redistribution during pregnancy (e, f, k, and l). Addition of LPS, which activates the HMs, did not affect the intracellular distributions of 6-PGDase, G-6-Pase, and LDH. Magnification, ×760. a–d and g–j: n = 16; e, f, k, and l: n = 5, where n is the number of patients contributing cells for these in vitro analyses.

** FIGURE 2.** Localization of enzyme activities in neutrophils. Representative cells from nonpregnant women (top row; a–f) and pregnant women (bottom row; g–l) are shown. Neutrophils were studied with and without prior incubation with LPS (50 ng/ml, 20 min). Cells were fixed then stained for 6-PGDase (a, b, g, and h), G-6-Pase (c, d, i, and j), and LDH (e, f, k, and l) activity (26, 27). 6-PGDase enzymatic activity is peripherally located in cells from nonpregnant women (a and b), but was found near the center of cells from pregnant women (g and h). Thus, 6-PGDase activity, in addition to its antigenic epitopes, undergoes differential trafficking in cells from nonpregnant and pregnant women. Positive and negative controls were performed using G-6-Pase activity and LDH activity, as in Fig. 1. LPS did not affect the intracellular distributions of 6-PGDase, G-6-Pase, and LDH. Thus, 6-PGDase activity parallels the distribution of G-6-Pase Ag identified in the immunofluorescence experiments above. Magnification, ×760. a–d and g–j: n = 12; e, f, k, and l: n = 5.
Colchicine disrupts 6-PGDase trafficking in pregnancy neutrophils

The ability of 6-PGDase to undergo either anterograde or retrograde distribution in cells under differing physiological conditions suggests that a component of the cytoskeleton is capable of actively translocating 6-PGDase within a cell. To test this concept, immunofluorescence localization of metabolic enzymes was performed on cells from pregnant and nonpregnant women in the presence and absence of colchicine, a microtubule-disrupting drug. Colchicine (50 μg/ml for 30 min at 37°C) had no effect on the intracellular distributions of 6-PGDase, G-6-PDase, and LDH of neutrophils from nonpregnant women (Fig. 4, a–f). However, when cells from pregnant women were treated with colchicine, the intracellular distribution of 6-PGDase became more normalized with staining associated with the MTOC, cytoplasm, and cell periphery (Fig. 4h). As a positive control, similar changes were noted for G-6-PDase (Fig. 4, c, d, i, and j). As a negative control, the intracellular distribution of LDH in neutrophils from pregnant and nonpregnant women in the presence and absence of colchicine was evaluated (Fig. 4, e, f, k, and l). The LDH distribution was not affected by colchicine. The ability of colchicine to disrupt the intracellular distribution of 6-PGDase suggests that its localization within pregnancy neutrophils is dependent upon microtubules.

6-PGDase traffics to the MTOC in cells from pregnant women

The fluorescence micrographs of Figs. 1g and 4g suggest that 6-PGDase undergoes retrograde motion in neutrophils from pregnant women, in contrast to the anterograde distribution within cells from nonpregnant individuals. To further test this concept, cells were labeled using direct immunofluorescence with FITC-conjugated anti-γ-tubulin and TRITC-conjugated anti-6-PGDase. γ-Tubulin is specific for MTOCs, which include the centrosome of interphase cells, polar bodies of mitotic cells, and basal bodies of flagella (33). Representative micrographs of neutrophils from pregnant and nonpregnant women that were fixed, extracted, and stained with FITC-anti-γ-tubulin and TRITC-anti-6-PGDase are shown in Fig. 5. Not surprisingly, anti-γ-tubulin decorated the centrosome (Fig. 5, b and e). When cells from pregnant women were stained with anti-γ-tubulin and anti-6-PGDase, the FITC-anti-γ-tubulin and TRITC-anti-6-PGDase patterns overlapped substantially (Fig. 5, d with e), which was not observed using cells from nonpregnant women (Fig. 5, a and b). Similar effects were noted for G-6-PDase (c, d, i, and j). Colchicine had no discernible effect on cells from nonpregnant women (a and b).
nonpregnant women (a and b). Although the similarity of the staining patterns in Fig. 5, d and e, suggests an association of γ-tubulin and 6-PGDase, it cannot assess the molecular proximity of these proteins. To detect molecular proximity of γ-tubulin and 6-PGDase, the technique of RET was used. Fluorescent labels must be within ~7 nm to obtain positive RET signals. We chose anti-6-PGDase as the acceptor label, because 6-PGDase is more abundant than γ-tubulin, thereby maximizing the RET signal. RET was studied using RET microscopy to determine the spatial locations of molecular proximity within cells, and emission microspectrophotometry to quantitatively measure the spectral intensities. As expected, RET was not detected between these labels on neutrophils from nonpregnant women using optical imaging (Fig. 5c) or spectrophotometry (Fig. 6e). However, RET imaging of pregnancy neutrophils indicates that RET is present at the MTOC (Fig. 5f), which is confirmed by spectrophotometry studies (Fig. 6g). These results indicate that 6-PGDase traffics to the MTOC in cells from pregnant women. Furthermore, 6-PGDase is within molecular proximity (~7 nm) of γ-tubulin in neutrophils from pregnant women, but not in cells from nonpregnant women.

The ability of colchicine to break up microtubules and normalize the intracellular distribution of 6-PGDase suggests that it should also reduce the amount of RET between 6-PGDase and γ-tubulin. Colchicine had no effect on the emission properties of cells labeled with only donor or acceptor labels (Fig. 6, a–d). Neutrophils from pregnant and nonpregnant individuals were treated with 50 μg/ml colchicine for 30 min at 37°C. Although colchicine treatment had no effect on cells from nonpregnant women (Fig. 6, e and f), colchicine exposure significantly reduced RET between 6-PGDase and γ-tubulin in cells from pregnant women (g vs h).

**6-PGDase and G-6-PDase form a supramolecular complex in neutrophils**

The parallel trafficking of 6-PGDase and G-6-PDase and their sensitivities to colchicine suggest that they may form a molecular complex on microtubules. RET experiments were performed to test this idea. Neutrophils from pregnant and nonpregnant women were stained with FITC-anti-G-6-PDase and TRITC-anti-6-PGDase as described above. Cells from nonpregnant women exhibited peripheral staining of 6-PGDase and G-6-PDase (Fig. 7, a and b). Importantly, RET imaging showed that these two enzymes exhibited molecular proximity (Fig. 7c). RET is also indicated by the emission spectroscopy results of Fig. 8 in comparison with that of a. When neutrophils from pregnant women were evaluated, RET between these two HMS enzymes were found at the MTOC.

**FIGURE 6.** RET emission spectrophotometry studies of 6-PGDase and γ-tubulin in neutrophils. Emission spectra were recorded for cells labeled with FITC only (a and b), TRITC only (c and d), both labels associated with cells from nonpregnant women (e and f), and both labels for cells from pregnant women (g and h). In some experiments, cells were incubated with colchicine (50 μg/ml for 30 min at 37°C) (b, d, f, and h). The fluorescence emission spectra of FITC (a and b) and TRITC (c and d) were not affected by colchicine. RET was not observed for cells from nonpregnant women (e and f). However, RET was observed for cells from pregnant women in both the absence (g) and presence (h) of colchicine. n = 7.
women in both the absence and presence of colchicine (Fig. 8f and h). RET was observed for cells from nonpregnant and pregnant women (Fig. 9, c and f). Similarly, RET was not observed in neutrophils from nonpregnant and pregnant women when labeled with anti-6-PGDase and anti-hexokinase reagents (data not shown). These findings suggest that some specificity is observed in the molecular proximity relationships formed among metabolic enzymes. Hence, 6-PGDase and G-6-PDase exhibit molecular proximity in cells from both pregnant and nonpregnant women.

We next sought to better understand the nature of the 6-PGDase and G-6-PDase complexes. For example, 6-PGDase and G-6-PDase could simply be brought together, because they both bind to microtubules or they could be assembled independently of microtubule structures. To ascertain the role of microtubules in 6-PGDase/G-6-PDase complex assembly, cells were treated with colchicine at 50 µg/ml for 30 min at 37°C. RET was observed using emission spectrophotometry (Fig. 8, f and h) and microscopic imaging (Fig. 10, c and f) in both the presence and absence of colchicine, which suggests that their proximity is not colchicine sensitive.

**Pregnancy alters the dynamic production of NAD(P)H**

Because 6-PGDase produces NADPH, we considered that 6-PGDase translocation may affect the nature of NADPH production. Previous studies have shown that the metabolism of neutrophils oscillates in time, and that the amplitudes and frequencies of these oscillations vary with exposure to activating stimuli (30, 34). Both experimental and theoretical studies indicate that NADPH oscillations are demodulated by living cells to yield oscillations in ROM production (34, 35). Fig. 11 shows real-time microfluorometry experiments of NADH → NADPH (NAD(P)H) autofluorescence in living cells as a function of time. Adherent neutrophils from nonpregnant and pregnant women were continuously analyzed during additions of FMLP and melatonin. Untreated cells from nonpregnant individuals displayed low-amplitude oscillations with a 20-s period. The period of these oscillations is reduced to ~10 s after addition of 0.5 µM FMLP. Melatonin promotes electron trafficking between the NADPH oxidase and myeloperoxidase and increases the amplitude of NAD(P)H oscillations (35). In contrast to IFN-γ, which requires 1–2 h to increase metabolic amplitudes, melatonin acts immediately and is therefore the preferred reagent in these real-time studies. When melatonin is added at 150 µg/ml, the oscillations are dramatically increased in amplitude. In contrast to the variable level of metabolic stimulation seen in cells from nonpregnant women, an intermediate behavior is observed for cells from pregnant women under all conditions; FMLP and melatonin had no effect on metabolic oscillations (Fig. 11). Thus, cell metabolism is unresponsive to different types of activating stimuli, which parallels a previous report from our laboratory using other activating agents (23).

![Figure 8](http://www.jimmunol.org/)

**FIGURE 8.** RET spectrophotometry studies of 6-PGDase/G-6-PDase complexes in neutrophils. Control studies with FITC only (a and b) and TRITC only (c and d) are shown. Experiments were performed by incubating cells without (a, c, e, and g) or with colchicine (50 µg/ml for 30 min at 37°C) (b, d, f, and h). RET was observed for cells from nonpregnant women in both the absence and presence of colchicine (e and f). However, RET was not intense (see arrows). RET was also observed for cells from pregnant women in both the absence (g) and presence (h) of colchicine. n = 4.

![Figure 9](http://www.jimmunol.org/)

**FIGURE 9.** 6-PGDase and LDH do not exhibit RET in both neutrophils from nonpregnant (a–c) and pregnant (d–f) women. Cells were fixed and then stained with FITC-conjugated anti-G-6-PDase and TRITC-conjugated anti-LDH. Although 6-PGDase and LDH are found at the periphery of nonpregnancy neutrophils (a and b), they do not exhibit RET, which suggests that they are not in close proximity. These different localizations in pregnancy cells is consistent with the absence of RET. Magnification, ×820. n = 3.

![Figure 10](http://www.jimmunol.org/)

**FIGURE 10.** RET between 6-PGDase and G-6-PDase is observed in neutrophils from pregnant women in the presence of colchicine. Cells were fixed and then stained with FITC-conjugated anti-γ-tubulin and TRITC-conjugated anti-6-PGDase. In the absence of colchicine, MTOC labeling was observed (a–c). As suggested by the spectrophotometry results of Fig. 7h, RET is observed in the presence of colchicine (f), although both enzymes have undergone dramatic redistributions due to colchicine treatment. Magnification, ×820. n = 4.
Pregnancy affects the production of oxidants

The effect of pregnancy on ROM and RNI production was evaluated. In the first series of experiments, we confirmed our recent observation that pregnancy neutrophils cannot be properly activated (23). Fig. 12 shows the rates of ROM and NO production by neutrophils from pregnant and nonpregnant women. In cells from nonpregnant women, low basal rates of ROM production are observed (Fig. 12a, trace 1), which are increased by exposure to IFN-γ (10 μg/ml for 1 h at 37°C) or to LPS (50 ng/ml for 20 min) (trace 3). Maximal levels of the ROM production rate are observed when IFN-γ pretreatment is combined with LPS addition (Fig. 12b, trace 4). In contrast, ROM production was at an intermediate value for ~75% of pregnancy neutrophils under all conditions (Fig. 12c) (see also Ref. 23). NO production was also evaluated (Fig. 12, b and d). Neutrophils from nonpregnant women display low levels of NO release (Fig. 12b, trace 1), which can be increased by incubation with IFN-γ (trace 2) or LPS (trace 3) and further increased by their combination (trace 4), as described above. Neutrophils from pregnant women displayed an intermediate slope that was not influenced by IFN-γ, LPS, or both of these reagents (Fig. 12d). Because both ROM and RNI production are powered by the same metabolic apparatus, it is not surprising that parallel observations concerning ROM and NO production were obtained.

Discussion

Pregnancy is a unique immunological state characterized by changes in both the adaptive and innate immunological responses (36, 37). One key element of both the innate and adaptive responses is the ability of leukocytes to generate ROMs and RNIs in response to opsonized and unopsonized pathogens. Neutrophils from pregnant women have been reported to have depressed ROM production (e.g., Ref. 2) and enhanced ROM production (38). A recent study from this laboratory (23) has suggested that the basal level of ROM production by pregnancy neutrophils is enhanced relative to unstimulated cells from nonpregnant women, whereas they cannot undergo activation to the same level as neutrophils from nonpregnant women. Thus, pregnancy neutrophils have an intermediate level of ROM production. A key factor limiting ROM production appears to be the translocation of G-6-PDase from the cell periphery to the MTOC in pregnancy neutrophils (23). We have proposed that the availability of the substrate NADPH is a key element in regulating NADPH and subsequent ROM production (e.g., Refs. 23, 34, 35, 39). Thus, NADPH oxidase assembly provides for a course regulation of its activity, whereas NADPH availability, including the spatial and temporal components of its production, provide a fine-tuning mechanism.

Our colocalization and RET experiments have shown that 6-PGDase accumulates at the MTOC and is in close physical proximity with γ-tubulin in neutrophils from pregnant women, but not nonpregnant women. This suggests that 6-PGDase undergoes retrograde trafficking during pregnancy and anterograde motion in cells from nonpregnant individuals, which parallels our recent work on G-6-PDase (23). Furthermore, the molecular proximity of 6-PGDase to cytoskeletal components is consistent with the ability of hexokinase, aldolase, PFK, GAPDH, and pyruvate kinase, to bind to microfilaments and/or microtubules (40–45). Thus, the intracellular trafficking of HMS and other cytoskeleton-associated metabolic enzymes allows neutrophils to vary HMS activity relative to glycolysis.

Enzyme-enzyme interactions have been demonstrated in several metabolic pathways, such as glycolysis and the tricarboxylic acid cycle. The formation of enzyme complexes allows the products of one enzyme to be directly passed to the next enzyme of the pathway without being released into the aqueous phase, thereby increasing efficiency. The first two steps of the HMS, which are catalyzed by G-6-PDase and 6-PGDase, constitute the primary source of NADPH production in cells. The present study has demonstrated RET between 6-PGDase and G-6-PDase, thus indicating that these enzymes are within ~7 nm of each other. The proximity of these two enzymes suggests that they form a supramolecular complex or metabolon within cells. This suggestion is in agreement with a previous biochemical study in plant and yeast systems using radiolabeled substrates that showed substrate channeling in the HMS (46). Hence, supramolecular complex formation explains...
why 6-PG formed by G-6-PDase does not equilibrate with 6-PG in the aqueous phase. However, it is not clear whether the molecular proximity of 6-PGDase and G-6-PDase was a result of enzyme-enzyme complex formation or simply the fact that both enzymes clustered together at the MTOTC of pregnancy neutrophils or at the periphery of cells from nonpregnant women. In other words, substrate channeling could be due to enzyme-microtubule interactions that lead to the molecular proximity of 6-PGDase and G-6-PDase. When cells from pregnant women were incubated with colchicine, 6-PGDase and G-6-PDase underwent dramatic redistribution within cells, but a substantial amount of RET remained, indicating that these enzymes were in the molecular proximity of each other independently of microtubules. Thus, our studies provide new structural evidence for the formation of supramolecular complexes of 6-PGDase and G-6-PDase. Furthermore, this finding is consistent with the fact that colchicine does not decrease the amount of superoxide produced (23), which would have been expected if microtubules were required for substrate channeling.

Our work suggests that 6-PGDase and G-6-PDase form a supramolecular complex in cells, which facilitates the production of NADPH by the HMS. In nonpregnant women, the complex is found in an anterograde distribution at the cell periphery. The peripheral distribution of the 6-PGDase/G-6-PDase complex facilitates its coupling with hexokinase, thereby promoting NADPH production. However, neutrophils from pregnant women are characterized by retrograde motion in a colchicine-sensitive (microtubule) manner; this is readily available to G-6-PDase/6-PGDase complex, thereby promoting NADPH production. This enzyme-microtubule interaction explains why 6-PG formed by G-6-PDase does not equilibrate with 6-PG in the aqueous phase, whereas the NAD(P)H concentration (34, 35, 39). Furthermore, we experimentally showed that ROM and NO production in activated neutrophils are linked with signal transduction/metabolic oscillations. The HMS plays a key role in the synthesis of ribose 5-phosphate, which is required for cell proliferation, and in NADPH production, which participates in biosynthetic pathways and in superoxide and NO production. The physical uncoupling of the 6-PGDase/G-6-PDase complex from peripheral cellular metabolism reduces the efficiency of NADPH production, at least under conditions of normal glucose concentrations. Consequently, reduced NADPH availability decreases superoxide production by the NADPH oxidase and NO synthesis by the NO synthase. This is quite reasonable given the observation that superoxide and NO production oscillate in both time and space with the intracellular NAD(P)H concentration (34, 35, 39). Furthermore, we experimentally showed that ROM and NO production in activated neutrophils was reduced in cells from pregnant women in comparison with activated cells from nonpregnant individuals. To our knowledge, this is the first time that pregnancy-associated changes in NO production have been reported. The reduction in oxidant production by cells from pregnant women may help to minimize oxidative damage to the conceptus (47).

Several additional implications of regulatory enzyme translocation might also be considered. Inasmuch as immunoregulation is an important aspect of pregnancy and because oxidant stress has been associated with several pregnancy-related clinical conditions (38, 47), the evaluation of G-6-PDase or 6-PGDase translocation may provide a novel means of monitoring pregnancy. We are presently studying the mechanism promoting retrograde G-6-PDase/6-PGDase complex transport in pregnancy neutrophils. If this normal physiological pathway could be understood, it might be possible to use this information as a route to develop novel anti-inflammatory compounds that provide similar functional changes in leukocytes.

References


