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Transaldolase is part of a supramolecular complex containing glucose-6-phosphate dehydrogenase in human neutrophils that undergoes retrograde trafficking during pregnancy

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Abstract

Previous studies have shown that glucose-6-phosphate dehydrogenase (G6PDase) and 6-phosphogluconate dehydrogenase form a supramolecular complex in human neutrophils that undergoes retrograde trafficking in cells from pregnant women, but anterograde trafficking in cells from nonpregnant individuals. Using fluorescence resonance energy transfer techniques, we now demonstrate that transaldolase (TALase), a key regulatory enzyme in the nonoxidative branch of the hexose monophosphate shunt, is in close physical proximity with G6PDase, but not with lactate dehydrogenase, thus suggesting the formation of a TALase-G6PDase complex. Moreover, immunofluorescence microscopy demonstrated that TALase undergoes anterograde trafficking in neutrophils from nonpregnant individuals, whereas retrograde trafficking is found during pregnancy. However, pregnancy did not affect lactate dehydrogenase distribution. Colchicine treatment blocked the retrograde distribution of TALase, suggesting that microtubules are involved in TALase trafficking. We suggest that TALase is part of a supramolecular hexose monophosphate shunt complex, which likely increases the efficiency of the shunt via substrate channeling. We further suggest that TALase's retrograde motion contributes to uncoupling the shunt from its source of glucose-6-phosphate at the plasma membrane, thereby blunting nicotinamide adenine dinucleotide phosphate (reduced form) production and downstream oxidant production by neutrophils. Published by Elsevier Inc.

1. Introduction

Pregnancy is a unique immunologic state characterized by alterations in a mother's inflammatory program. These changes include reductions in neutrophil chemotaxis, superoxide and reactive oxygen metabolite (ROM) production, and phagocytosis in comparison with those of nonpregnant women [1-4]. These cell biological changes may contribute to the increased susceptibility of pregnant women to certain infections such as *Listeria monocytogenes*, *Toxoplasma gondii*, *Neisseria gonorrhoeae*, and *Plasmodium vivax* [5-8]. Furthermore, the reduced proinflammatory capacity of pregnant women may contribute to the remission of autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, and uveitis observed in roughly 70% of these women [9-11]. Although normal pregnancy neutrophils do not undergo complete activation, their basal levels of ROM production are higher than those from nonpregnant women [12]. Hence, pregnancy is a complex physiological state that provides insights into the regulatory mechanisms of neutrophil activation.

One robust measure of neutrophil and monocyte activation is the production of superoxide anions, which play a key role in a host's oxidative defense as well as tissue damage in several clinical settings [13]. Superoxide formation begins with glucose entry, which is required for its production [14-16]. Superoxide is synthesized by the enzyme nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) oxidase according to the relationship

$$NADPH + 2O_2 \rightarrow NADP^+ + H^+ + 2O_2^-.$$

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A cell's primary source of NADPH is the oxidative branch of the hexose monophosphate shunt (HMS), whose substrate is glucose 6-phosphate (G6P) (Fig. 1). Glucose 6-phosphate is generally supplied by hexokinase upon glucose entry, although it can also be produced by running the phosphohexose isomerase "backward" from fructose 6-phosphate (F6P) to G6P. The first step of the HMS is the irreversible and highly regulated conversion of G6P to NADPH and 6-phosphogluconolactone by glucose-6-phosphate dehydrogenase (G6PDase). This is then converted by 6-phosphogluconate dehydrogenase (6PGDase) into ribulose 5-phosphate and another NADPH molecule. Ribulose 5-phosphate can be used for the synthesis of nucleic acids via ribose-5-phosphate, or it can enter the HMS's nonoxidative branch, which creates a reversible link with glycolysis. Transaldolase (TALase) is a 37.5-kd enzyme that influences the division of cell resources among NADPH, adenosine triphosphate (ATP), and nucleic acid production. Transaldolase is a key regulatory enzyme [17,18] of the HMS's nonoxidative branch that reversibly transfers a 3-carbon ketol from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate (GA3P) to form F6P and erythrose 4-phosphate. Together with transketolase, these enzymes link the HMS with glycolysis. Furthermore, GA3P can directly enter glycolysis to provide more ATP or enter gluconeogenesis to return to the shunt to yield more NADPH. Fructose 6-phosphate produced by the shunt's nonoxidative branch can be converted to G6P by the reversible phosphohexose isomerase, which allows carbon chains to reenter the shunt's oxidative arm. Although no NADPH is produced in the nonoxidative stage, it balances the oxidative and nonoxidative stages of HMS and determines the efficiency of NADPH production by directing the flux of carbon atoms.

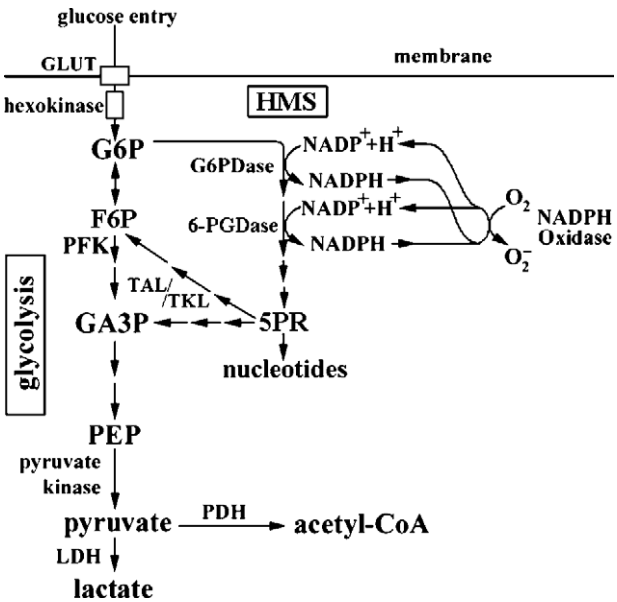

Fig. 1. Glycolysis and the HMS. Important enzymes shown here are G6PDase, 6PGDase, TALase, NADPH oxidase, and LDH. The substrates listed here are G6P, ribulose 5-phosphate, GA3P, and NADPH. 5PR indicates ribulose 5-phosphate.

We have recently discovered a novel mechanism regulating HMS activity that involves the intracellular-trafficking G6PDase, which, in parallel, regulates ROM production [12]. This regulatory mechanism is apparently specific for pregnant women. In leukocytes from nonpregnant women and men, G6PDase and 6PGDase are located at the cell periphery where the substrate G6P is produced and is therefore readily available to the shunt. However, in cells from pregnant women, G6PDase and 6PGDase are transported in a retrograde fashion to a cell's microtubule-organizing center (MTOC) [12,19], where G6P is less available, thereby limiting HMS activity. Thus, the spatial positions of enzymes may play a key role in clinical phenotype without changing the total expression level of an enzyme. Because TALase is a key enzyme regulating the recycling of carbon from and to the shunt and therefore the total NADPH production, we hypothesize that TALase may be a part of the supramolecular complex containing G6PDase and 6PGDase that undergoes retrograde trafficking during pregnancy.

2. Materials and methods

2.1. 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 weeks to term. Eligible patients were approached at the Detroit Medical Center/Wayne State University (Detroit, Mich).

2.2. Cell preparation

Neutrophils were isolated from blood samples using Ficoll-Hypaque (Sigma-Aldrich, St Louis, Mo) density gradient centrifugation [12]. Neutrophil viability was more than 95% as assessed by trypan blue exclusion. Cells were suspended in Hanks balanced salt solution (HBSS; Life Technologies, Grand Island, NY).

2.3. Reagents and antibodies

Colchicine, lipopolysaccharide (*Escherichia coli*, serotype 026:B6), and *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) were obtained from Sigma-Aldrich. Fluorescein isothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRITC) were obtained from Molecular Probes (Eugene, Ore). Rabbit anti-G6PDase and goat anti–lactate

dehydrogenase (LDH) polyclonal antibodies (Abs) were obtained from Chemicon International (Temecula, Calif). Antitubulin was obtained from R&D Systems (Minneapolis, Minn). Anti-TALase was obtained from Nordic Immunological Laboratories (Tilburg, the Netherlands). Fluorescein isothiocyanate– and tetramethylrhodamine isothiocyanate–conjugated Abs were prepared as described [20].

2.4. Immunofluorescence staining

Neutrophils were placed on glass coverslips, incubated with reagents as described hereinafter, and then fixed with the protocol of Naftalin [21]. Briefly, cells were fixed with 2% paraformaldehyde, permeabilized with 1% Brij-58, and fixed with 2% paraformaldehyde at room temperature for 20 minutes. Cells were washed with HBSS, labeled with 1 μg/mL of FITC- and/or TRITC-conjugated Abs at 4°C for 30 minutes and then washed again with HBSS at room temperature.

2.5. 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 (Frederick, Md) [22]. A narrow bandpass discriminating filter set (Omega Optical, Battleboro, Vt) 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 dichroic mirrors of 510 and 560 nm were used for FITC and TRITC, respectively. For fluorescence resonance energy transfer (FRET) imaging, 485/22 and 590/30 nm optical filters were used for excitation and emission, respectively, in conjunction with a 510-nm dichroic mirror. The fluorescence images were collected with an intensified charge-coupled device camera (Princeton Instruments, Princeton, NJ).

2.6. Single-cell emission spectrophotometry

Energy transfer was also examined by means of a microscope spectrophotometer apparatus [23,24]. 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 dichroic mirror, and a 520-nm long-pass emission filter. Winspec software (Princeton Instruments) was used to analyze spectrophotometric data.

3. Results

3.1. Transaldolase trafficking in neutrophils from pregnant women

We have recently reported that the enzymes G6PDase and 6PGDase form a supramolecular complex in peripheral blood neutrophils that undergoes differential trafficking during pregnancy [19]. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase undergo anterograde trafficking in neutrophils from nonpregnant women, but retrograde trafficking in maternal neutrophils [12,19]. Here, we examine the intracellular distribution of TALase, another key HMS enzyme, using immunofluorescence microscopy. As illustrated in Fig. 2, TALase is found primarily at the periphery of neutrophils from nonpregnant women in both stimulated (fMLP at 0.1 μmol/L) and untreated groups (Fig. 2A and B). However, TALase is translocated to the MTOC (or centrosome) in cells from pregnant women in the presence or absence of fMLP (Fig. 2G and H). As a positive control, parallel changes were observed for G6PDase (Fig. 2C, D, I, and J) under identical conditions. However, no differences were observed for LDH trafficking, which constitutes a negative control (Fig. 2E, F, K, and L). No changes in the localization of other metabolic enzymes, including phosphofructokinase and pyruvate kinase, were reported previously [12]. Thus, pregnancy, but not fMLP stimulation, affects TALase trafficking in neutrophils.

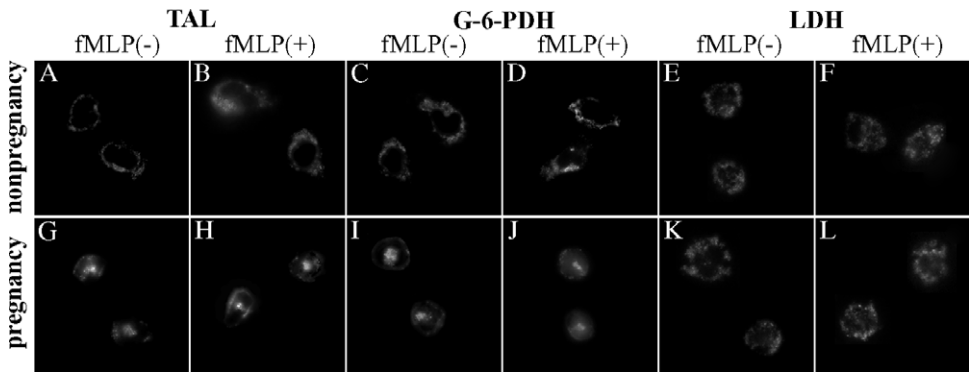

Fig. 2. Immunofluorescence microscopic studies of TALase, G6PDase, and LDH in neutrophils. Representative data of cells stimulated with or without fMLP (0.1 μmol/L) from nonpregnant women (A-F, top row) and pregnant women (G-L, bottom row) are shown. Cells were fixed then stained with FITC–anti-TALase (A, B, G, and H), TRITC–anti-G6PDase (C, D, I, and J), and FITC–anti-LDH (E, F, K, and L) (original magnification ×760; n = 4, where n is the number of patients contributing cells for these in vitro analyses).

3.2. Formation of a supramolecular complex containing TALase and G6PDase

The parallel intracellular trafficking of TALase and G6PDase suggests that TALase may be a part of the G6PDase-6PGDase supramolecular complex of HMS enzymes. Fluorescence resonance energy transfer experiments, which detect molecular proximity, were performed to test this concept. Neutrophils from pregnant and nonpregnant women were stained with FITC–anti-TALase and TRITC–anti-G6PDase as described previously. Cells from nonpregnant women exhibited peripheral staining of TALase and G6PDase (Fig. 3A and B), and FRET imaging demonstrated that these 2 enzymes exhibited molecular proximity (Fig. 3C). Fluorescence resonance energy transfer is also confirmed by the emission spectroscopy results of Fig. 4C (solid line). When neutrophils from pregnant women were evaluated, FRET between these 2 HMS enzymes was found at the MTOC (Fig. 3F). Fluorescence resonance energy transfer was also observed during emission spectroscopy experiments Fig. 4E, solid line). However, FRET was not observed between FITC–anti-LDH and TRITC–anti-TALase in neutrophils from nonpregnant or pregnant women in either imaging (Fig. 3I and L) or emission spectroscopy (Fig. 4D and F, solid lines) experiments. As FRET is observed only when 2 molecules

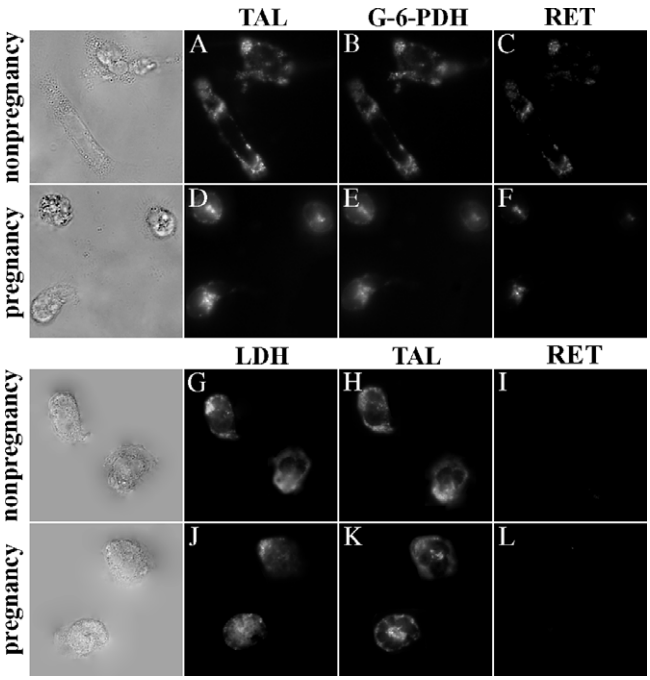

Fig. 3. Detection of FRET between TALase and G6PDase or LDH. Cells from nonpregnant (A-C and G-I) or pregnant (D-F and J-L) women were fixed then double-stained with FITC–anti-TALase and TRITC–anti-G6PDase or with FITC–anti-LDH and TRITC–anti-TALase as described in Materials and methods. Fluorescence resonance energy transfer between TALase and G6PDase is shown in panels A to F, and the lack of FRET between TALase and LDH labels is confirmed in panels I and L (original magnification ×820; n = 5).

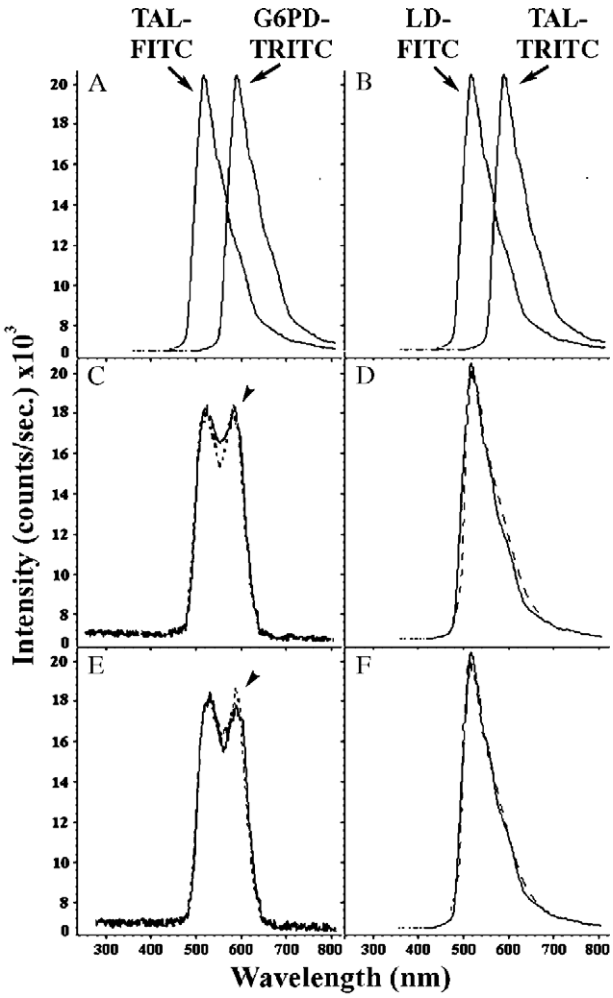

Fig. 4. Emission spectrophotometry studies of the physical proximity of TALase, G6PDase, and LDH. Neutrophils from nonpregnant (C and D) or pregnant women (E and F) were treated with (dotted lines) or without (solid lines) colchicine (50 μg/mL) at 37°C for 30 minutes. Cells were then fixed and double-stained with FITC–anti-TALase and TRITC–anti-G6PDase (A, C, and E) or with FITC–anti-LDH and TRITC–anti-TALase (B, D, and F). For comparison, single staining with FITC- or TRITC-conjugated Abs are shown in panels A and B. Fluorescence resonance energy transfer is indicated by arrowheads (n = 3).

are within 7 nm of one another [25], these findings suggest that TALase and G6PDase are physically associated within neutrophils from pregnant and nonpregnant women.

3.3. Colchicine disrupts retrograde TALase trafficking in maternal neutrophils but does not effect TALase-G6PDase proximity

The observed differences in TALase trafficking during pregnancy suggests the involvement of cytoskeletal components. A G6PDase-6PGDase supramolecular complex has been previously reported to be colocalized with microtubule components in maternal neutrophils [12,19]. To ascertain the role of microtubules in TALase-G6PDase complex properties, the microtubule-disrupting reagent colchicine was used. As shown in Fig. 5, treatment of

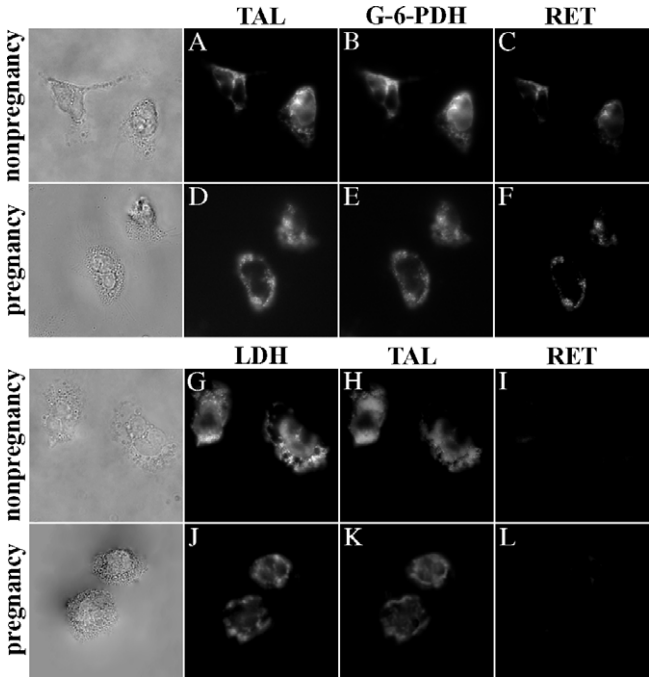

Fig. 5. Effect of colchicine on intracellular localization of TALase, G6PDase, and LDH. Neutrophils from nonpregnant (A-C and G-I) or pregnant women (D-F and J-L) were incubated with colchicine (50 μg/mL) at 37°C for 30 minutes. Cells were fixed then double-stained with FITC–anti-TALase (A and D) and TRITC–anti-G6PDase (B and E) or with FITC–anti-LDH (G and J) and TRITC–anti-TALase (H and K). Fluorescence resonance energy transfer was observed between TALase and G6PDase (C and F) but not for the TALase and LDH labels (I and L) (original magnification ×820; n = 4).

cells with colchicine (50 μg/mL for 30 minutes at 37°C) did not alter TALase (Fig. 5A) and G6PDase (Fig. 5B) localization in neutrophils from nonpregnant women, but disrupted the MTOC trafficking of these enzymes in maternal cells (Fig. 5D and E). However, colchicine treatment did not effect the physical association of TALase with G6PDase in neutrophils from either nonpregnant (Fig. 5C) or pregnant women (Fig. 5F). Similar results were obtained in emission spectroscopy experiments (Fig. 4C and E, dotted lines). As a negative control, the effect of colchicine on intracellular LDH trafficking and association with TALase has also been examined. Colchicine did not affect LDH localization in neutrophils from nonpregnant (Fig. 5H) or pregnant women (Fig. 5K), and there was no FRET detected between TALase and LDH (Figs. 4D, F and 5I, L) with or without colchicine treatment. Thus, the retrograde trafficking of TALase in maternal neutrophils depends on microtubules, but the formation of TALase-G6PDase supramolecular complexes does not rely on microtubules and is not influenced by pregnancy.

4. Discussion

Although the basal level of extracellular ROM release is enhanced during pregnancy, normal maternal neutrophils from healthy women are refractory to further stimulation in vitro because they fail to respond to numerous activating substances [1-4,12,19]. For example, cell stimulation does not lead to an increase in ROM or nitric oxide production (eg, 2, 12, and 19), although higher levels of ROM production are observed in certain disease states in pregnancy [26]. We have previously emphasized that the NADPH oxidase is regulated not only by signaling events such as phosphorylation but also by the availability of its substrate NADPH, which is chiefly produced by the HMS. The HMS, in turn, is regulated by the availability of its substrate, G6P. Glucose 6-phosphate availability and, indeed, metabolism in general are controlled largely by the rate of glucose flux [27]. An additional means of regulating G6P availability to the shunt is by spatially separating hexokinase, which traffics to the cell periphery and produces G6P from G6PDase, the shunt's first step. We have recently discovered that this latter strategy is used by leukocytes from pregnant women. During pregnancy, the oxidative arm of the shunt (G6PDase and 6PGDase) undergoes retrograde translocation to the MTOC and therefore blunts HMS activity and ROM production [12,19]. Another key enzyme of HMS, TALase, is a component of the nonoxidative arm that influences total NADPH production. The present report demonstrates the retrograde trafficking of TALase in maternal neutrophils, which is not effected by fMLP stimulation. All of the crucial enzymes of HMS, including G6PDase, 6PGDase, and TALase, undergo parallel trafficking to the MTOC during pregnancy. Hence, the physical relocation of HMS enzymes to the MTOC appears to be a broad physiological strategy of pregnancy to regulate ROM production by maternal neutrophils.

Cytoskeletal components such as microfilaments and microtubules participate in transporting various intracellular components from place to place within cells. Metabolic enzymes, including hexokinase, aldolase, phosphofructokinase, and pyruvate kinase, may be attached to the cytoskeleton and in certain cases undergo translocation [21,28-33]. We have previously reported that microtubules, but not microfilaments, transport G6PDase and 6PGDase to the MTOC in cells from pregnant women [12,19]. In the present study, we have shown that TALase colocalizes with G6PDase at the MTOC in maternal neutrophils, TALase colocalizes with γ-tubulin of MTOCs in maternal neutrophils (data not shown), and treatment with colchicine, a microtubule-disrupting reagent, disrupts the localization of TALase to MTOCs. These studies suggest that microtubules mediate retrograde TALase trafficking during pregnancy.

Enzymes often form supramolecular complexes, which allow the products of one enzyme to be directly passed to the next enzyme without entering the aqueous phase, thereby increasing efficiency [34]. Our data indicate that TALase, in addition to G6PDase and 6PGDase, is physically associated to form a supramolecular structure, as judged by FRET techniques. Because the complex is not dissociated by the addition of colchicine, their proximity is

apparently not explained by their common association with microtubules. Because FRET was detected in cells from pregnant and nonpregnant women, the HMS assemblage must be a relatively stable structure that is simply moved during pregnancy.

We have emphasized the importance of depressing HMS activity to reduce the production of oxidative molecules, thereby lessening their potential damaging effects on mother and fetus. However, other advantages may accrue to the host. For example, during neutrophil activation by a wide spectrum of agonists, the HMS becomes activated. When the HMS is activated, carbon flux through glycolysis is necessarily decreased (at a constant glucose influx), and therefore, the resources available for ATP production are consequently decreased. By limiting cell activation and HMS activity during pregnancy, some NADPH remains available while maintaining high levels of ATP production.

It may seem paradoxical that the activity of the HMS is depressed during pregnancy. Rapid growth must be supported by the synthesis of nucleic acids, and nucleic acid synthesis is linked to the production of ribose-5-phosphate, which can be formed by the oxidative arm of the HMS. However, under conditions of high ribose-5-phosphate demand, carbon flow through TALase and transketolase will be in the opposite direction (from F6P to pentose phosphates [35]) to supply cells with the needed substrate. Therefore, producing NADPH at an intermediate level is not inconsistent with large amounts of ribose-5-phosphate formation.

In summary, the parallel trafficking of TALase with G6PDase and 6PGDase, the physical proximity of TALase with G6PDase, and the microtubule-dependent retrograde translocation of TALase, G6PDase, and 6PGDase in maternal cells all suggest that both the oxidative and nonoxidative arms of the HMS are associated in the same structural microcompartment. To blunt harmful ROM and nitric oxide production by neutrophils during pregnancy, the HMS complex is moved from the cell periphery that is rich in glycolytic activity by traveling along microtubules. This constitutes a novel physiological “shortcut” in the regulation of ROM production by maternal neutrophils. It will be interesting to identify the motor proteins and signaling pathways associated with the trafficking of the HMS complex. Further dissection of this pathway will lead to a better mechanistic understanding of the retrograde transport of the HMS enzyme complex in maternal neutrophils, which we believe may provide a route to develop novel anti-inflammatory compounds that provide similar metabolic changes in the leukocytes of all patients.

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