Journal of Reproductive Immunology 104–105 (2014) 26–31

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

Cell-free nucleic acids in (maternal) blood: any relevance to (reproductive) immunologists?

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ARTICLE INFO

Article history:
Received 10 December 2013
Received in revised form 19 March 2014
Accepted 25 March 2014

Keywords:
Cell-free DNA
Placenta
Neutrophil extracellular traps (NETs)
miRNA

ABSTRACT

Cell-free foetal DNA recently hit the international headlines by facilitating the non-invasive prenatal testing (NIPT) of foetal chromosomal anomalies directly from maternal blood samples. Being largely of placental origin, cell-free foetal DNA may also, however, provide insight into underlying pathological changes in preeclampsia, or the influences of external stresses, such as hypoxia. This analysis may be enhanced by the simultaneous assessment of placenta-derived, cell-free mRNA species. The source of maternal cell-free DNA is not readily apparent, but may involve neutrophil extracellular traps (NETs). The rapid rise in this material following removal of the placenta, especially in preeclampsia, may indicate a rapid transient maternal inflammatory response to placenta-derived debris. Since NETs have recently been shown to promote coagulation, this may provide a link to pregnancy-associated thrombosis or placental infarction. The presence of cell-free, placenta-derived DNA may not be as innocuous as commonly assumed, as it is largely hypomethylated and could, like bacterial DNA, trigger the activation of maternal immune effector cells via interaction with toll-like receptor 9 (TLR9), thereby contributing to an excessive inflammatory response in preeclampsia or preterm labour. Possibly the most fascinating aspect concerning placenta-derived, cell-free nucleic acids is the recent report that placental exosomes loaded with placenta-specific C19MC miRNA species may modulate the antiviral response of maternal immune cells, thereby ensuring foetal well-being.

1. Cell-free nucleic acids: more than just DNA

Even though the presence of DNA in the plasma and serum of humans had already been described in 1948 (Mandel and Metais, 1948), before the structure or role of DNA was determined (Watson and Crick, 1953), it is only in the past decade or two that there has been a resurgence of interest in this analyte (Lo and Chiu, 2011). This renewed interest was initially fuelled by the discovery of tumour-derived, cell-free DNA in cancer patients (Chen et al., 1996), a pivotal finding that provided the spark for the discovery of cell-free foetal DNA in maternal plasma and serum (Lo et al., 1997). This latter finding paved the way for the first clinical translation and commercial realization of cell-free nucleic acid analysis in the form of non-invasive prenatal testing (NIPT) for foetal chromosomal anomalies (Chiu and

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http://dx.doi.org/10.1016/j.jri.2014.03.007
0165-0378/

Lo, 2011; Hahn et al., 2012b; Hui and Bianchi, 2013). It also provided the impetus to examine the role of cell-free DNA in a wide variety of other pathological conditions ranging from pregnancy-related problems, such as preeclampsia, preterm labour and hyperemesis, to autoimmune disorders, sepsis, stroke, coagulopathies and sports physiology (Atamaniuk et al., 2008; Borissoff et al., 2013; Demers et al., 2012; Fuchs et al., 2010; Hahn et al., 2005; Holdenrieder and Stieber, 2009; Zhong et al., 2007).

The presence of cell-free nucleic acids in plasma, however, is not restricted to DNA, it includes mitochondrial DNA as well as messenger RNA (mRNA) and microRNA (miRNA) species (Chim et al., 2008; Lo and Chiu, 2004). The presence of these other nucleic acids permits a much broader array of questions to be addressed (Chim et al., 2008; Hahn et al., 2005; Lo and Chiu, 2011; Lo et al., 2007).

2. Cell-free DNA: source and underlying biology

The ability to determine foetal cell-free DNA concentrations with the Taqman® real-time PCR assays specifically for the Y chromosome permitted an assessment of changes in cell-free DNA concentrations during gestation (Lo et al., 1998a), as well as alterations in pregnancy-associated disorders, such as preeclampsia (Lo et al., 1999a; Zhong et al., 2001b). This technology also allowed the examination of post-partum concentrations. Surprisingly, cell-free DNA disappeared rapidly after delivery, with a half-life of approximately 15 min (Lo et al., 1998a, 1999b) (Fig. 1).

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This rapid kinetic change, however, is not restricted to foetal cell-free DNA species, it can also be exhibited by total cell-free DNA levels in normal individuals who have undergone physical exertion, for instance, participation in a marathon race (half and ultra). In the instance of a half marathon (21.09 km), total cell-free DNA increased approximately 18.5-fold in samples collected immediately post-finish line, and a rapid decrease to almost basal levels was noted in samples collected 2 h later (Atamaniuk et al., 2004). In the case of an ultra-marathon (100 km/6 h), once again an almost 18-fold increase in total cell-free DNA concentrations was noted in blood samples collected immediately post-finish line (Atamaniuk et al., 2008). However, in this instance, an almost three-fold elevation in cell-free DNA concentration was noted in samples collected 2 h later, with a reduction to basal levels only being attained after 24 h (Atamaniuk et al., 2008).

Although there was some debate over the source of cell-free foetal DNA, it is now well accepted that it is almost exclusively of trophoblast origin and not derived from the demise of trafficking foetal cells (Chiu and Lo, 2011; Zhong et al., 2002). This is due to:

i. The concentration of cell-free foetal DNA being much higher than the numbers of rare trafficking foetal cells.
ii. Elevations in cell-free foetal DNA concentrations can occur independently of changes in the trans-placental traffic of foetal cells.
iii. The rapid clearance of cell-free foetal DNA post-delivery, whereas trafficking foetal cells can persist post-partum.
iv. Cell-free foetal DNA sharing epigenetic features common to trophoblast cells.
v. The presence or absence of genetic loci in cell-free foetal DNA is affected by placental mosaicism.
vi. Cell-free "fetal" DNA is detectable in anembryonic pregnancies.
vii. Cell-free foetal DNA is associated with placenta-derived microparticles.

For this reason, it is probably more correct to refer to this material as cell-free "*placenta-derived*" DNA, rather than cell-free foetal DNA. Owing to the widespread popular use of cell-free foetal DNA, it is very likely that the latter terminology will persist (Chiu and Lo, 2011).

The source of total cell-free DNA in the circulation of normal individuals or pregnant women is more complex. While it is clear that solid organs, such as the kidney or liver, do contribute to the pool of total cell-free DNA, this represents only a minor fraction (Lo et al., 1998b; Zhong et al., 2001a), with the vast proportion of cell-free DNA appearing to be of haematopoietic origin (Lui et al., 2002). This would suggest that the source of this material would be the rapid turn-over of short-lived cells, such as neutrophils, or via the enucleation of erythroblasts as they mature to erythrocytes.

Furthermore, it appears that foetal and total cell-free DNA are derived by different modes, in that they differ in size, foetal fragments being significantly smaller than maternal ones, a feature confirmed by different technologies including agarose gel electrophoresis, real-time PCR and massive parallel sequencing (Hahn and Zimmermann, 2010). While foetal fragments are routinely of the order of 130–150 bp, the size of maternally derived molecules may be larger than anticipated, with reports indicating that they can be in excess of 23 kb (Li et al., 2004). In this context it is worth noting that the genomic DNA in erythroblast nuclei prior to expulsion is cleaved into very large mega base-pair fragments (Hristoskova et al., 2007). This would suggest that erythroblast enucleation might be a source of this material.

A surprising turn of events was the stunning observation in 2004 that neutrophils can expel their genomic DNA into the extracellular environment in the form of neutrophil extracellular traps (NETs) (Brinkmann et al., 2004). Since NETs are detected in preeclamptic placentae, it was hypothesized that NETs might contribute to the elevations in total cell-free DNA seen in this disorder (Gupta et al., 2006).

Independent support suggesting that neutrophil NETs might contribute to elevations in total cell-free DNA levels may be provided by the study of ultra-marathon runners, as here a significant increase in circulatory neutrophils was noted, with a return to basal levels 24 h after the event (Atamaniuk et al., 2008). Hence, these data suggest that neutrophil NETs contributed to the elevated pool of cell-free DNA observed in post-race plasma samples.

In summary, these data suggest that the steady-state pool of total cell-free DNA might be derived from erythropoiesis, whereas rapid elevations in cell-free DNA evident in inflammatory or physically strenuous conditions, may be the result of excessive NETosis.