

But the reverse is not true: a critical review on staining protocol of uterine natural killer cells!

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ABSTRACT

The staining protocol of uterine natural killer-cells (uNKs) used by some previous studies is a discussion-provoking issue through the lens of immunoassay. Most anatomists are of the conviction that uNKs are detectable by periodic acid Schiff (PAS) whereas most immunologists do not think so and they take CD markers as a gold standard to detect immune cells. So we intended to have a discussion on this issue and propose our critical viewpoints in the present critical review. Although PAS method is more appropriate than Haematoxylin and Eosin (H&E), but this method of staining is still general; because some other leukocytes are PAS positive too that we cannot recognize whether the arrowed cells are uNKs or other large granular cells. NK subpopulations, CD markers and killer-cell immunoglobulin-like receptors (KIR) types are the factors that are not possible to be detected by PAS staining. Although all NKs are PAS positive, but the reverse is not true!

KEY WORDS: NK cells, periodic acid Schiff, implantation.

1. INTRODUCTION

Infertility is a multi-factorial disorder and has a variety of pathogenesis (Yasin Ahmadi, 2015). Generally it can be caused by male or female complications consists of sperm abnormalities (Khaki, 2009; 2014; 2015), anti-sperm antibodies produced by women's immune system (Talebi Farahani, 2016), infectious disease and agents (Talebi Farahani, 2016; Kurdoglu, 2015; Garedaghi and Bahavarnia, 2014), genetic disorders (Tavukcuoglu, 2012; Dolatabad, 2014; Al-Azawi, 2013; Telli, 2014; Ranjbar, 2014), ovulation and implantation disorders (Biegy, 2003; Boroujeni, 2012; Boroujeni, 2010; Fayazi, 2014), reaction of women's immune system against the semi-allograft fetus (Ahmadi, 2016) and neoplastic conditions (Jafari-Shobeiri, 2016; Borna, 2012).

According to the items mentioned above, most infertilities have immune-based causes. Since natural killer-cells (NKs) are the first response immune cells in a lot of immune reactions and these cells play their role as a bridge between the innate and adaptive immune systems, they are notable (Zarnani, 2015; Ahmadi, 2016; Lash, 2016). The staining protocol of uterine NKs (uNKs) used in some previous studies like (Murphy, 2005), is discussion-provoking issue through the lens of immunoassay. Most anatomists (in particular embryologist and histologist) are of the conviction that uNKs are detectable by periodic acid Schiff (PAS) (Beigi Boroujeni, 2015), whereas most immunologists do not think so and they take CD markers as a gold standard to detect immune cells. The interesting point is that in the word of "The journal of immunology" (2005) the high capacity of Carbohydrate content of the perforin-containing granules of uNKs enable them to be visualized by staining with PAS. So we intended to have a discussion on this issue and propose our critical viewpoints.

2. METHODS

A brief critical review with a critical approach based on our experiences and previous publications.

3. RESULTS AND DISCUSSION

Although PAS method is more appropriate than Haematoxylin and Eosin (H&E), but this method of staining is still general; because some other leukocytes are PAS positive too that we cannot recognize that whether the arrowed cells are uNK or other large granular cells. Also we have a variety of NKs; e.g. most of the peripheral blood NKs are CD16⁺CD56^{dim} while most of the uNKs are CD16⁻CD56^{bright}; the dim form has more cytotoxic capacity called as "cytotoxic NK" and the bright form contributes in secretion of inflammatory cytokines (Rajaei, 2011; Chen, 2015) called as "immune-regulatory NK". The two types exist respectively mainly in blood and implantation site (table 1). So uNKs mostly play their roles in contrast to the connotation of their name (Shahsavari, 2011; Mousavi, 2009; Mousavi, 2011; Shahsavari, 2012; 2013; Ghafourian, 2015).

Table.1. The subsets of NKs.

NK type	Cytotoxic	Immune - regulatory
CD 16	+	-
CD 56	Dim	Bright
CD 94*	+	+++
KIR	+++	+
Cytokines releasing	Low	High
ADCC**	+	-

The minus mark means very low frequency (not its absence). * CD 94 is of the inhibitory coreceptors like KIR. **

ADCC stands for antibody dependent cell-mediated cytotoxicity which is performed through CD16.

In addition to the lack of ability to identify the CD markers by PAS staining, about 14 types of killer-cell immunoglobulin-like receptor (KIR) have been discovered so far on the surface of NKs (table 2) and rather in which KIR gene binds to which allelic type of human leukocyte antigen (HLA), the NKs perform different functions that most of them are protective for maintenance of pregnancy (immune-tolerance) (Ahmadi, 2016; Shahsavar, 2011). Thereby with PAS staining we cannot distinguish neither the lymphocyte type nor the CD markers and nor the KIR type.

Table.2. KIR genes

KIR genes	Inhibitory KIRs	Activating KIRs	Pseudo genes
	2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3	2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DS1	2DP1, 3DP1

KIR has 14 discovered genes and 2 discovered pseudo-genes. Eight number of them are inhibitory and 6 number them are activating. Each gene has different alleles; So KIR is highly polymorphic like HLA.

Since the study of Murphy et al (Murphy et al., 2005) had some monies-taking tests such as flow-cytometry, they were supposed to use more appropriate staining protocol such as cytokine staining (Moncunill, 2015), immunohistochemical staining (Wilgenburg, 2005) or immunofluorescent staining (Sanden, 2015). Of course we appreciate their perfect experimental skill that you can see a part of their picture in figure 1 as you see in the study of Boroujeni (2015) (both of them are sufficiently detectable, but it would better to use more specific technic). It would be better also to use gel-based (Mitra and Mandana, 2015) and/or molecular technics that as an instance Beigi Boroujeni staffed real-time polymerase chain reaction (PCR) to detect expression of vascular endothelial growth factor (VEGF) at implantation site (Beigi Boroujeni, 2016; Boroujeni, 2016).

4. CONCLUSION

At the end the present critical review we intend to say that the immune-regulatory subset of NKs are beneficial to improve fertility rate; therefore immune-based tests must have sufficient sensitivity to separate them from each other, because wrong diagnosis may lengthen treatment procedure and make it monies-taking and sometimes may lead to abortion secondary to wrongly prescribed drugs that destroy or decrease the natural profile of immune-regulatory NKs. Although all NKs are PAS positive, but the reverse is not true!

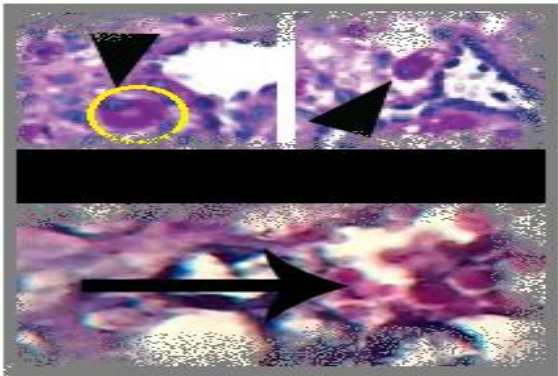


Figure.1. Parts of some figures from Murphy (2005) (above) and Boroujeni (2015) (below). the figures are graphically changed with gray color because of copyright policies.

5. ACKNOWLEDGEMENTS

The present paper is extracted from a research proposal supported by Student Research committee of Lorestan University of Medical Sciences.

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