

Monocyte cytokine production during pregnancy

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With great interest, we have read the article, "Monocytes are progressively activated in the circulation of pregnant women" [1]. In their article, the authors showed that there is progressive, generalized, inflammatory cell activation, characterized by progressive up-regulation of CD11a, CD54, and CD64 during pregnancy. In addition, granulocyte numbers were increased, and lymphocyte numbers decreased during pregnancy.

In this comprehensive paper, the authors not only showed phenotypical activation of the inflammatory cells but also functional activation of these cells, as they showed an increase in the percentage of monocytes spontaneously producing interleukin (IL)-12 in third-trimester pregnant women as compared with nonpregnant women and increased IL-1 β production by stimulated second-trimester pregnant monocytes as compared with stimulated, nonpregnant monocytes. Moreover, stimulated third-trimester pregnant granulocytes produced more IL-8 than nonpregnant granulocytes.

These data are in line with the hypothesis that pregnancy is a proinflammatory condition [2–4]. However, for adhesion molecule expression and intracellular cytokine production, not all data in this paper are consistent with data in other papers about the same subject [3–6], including a previous paper by Luppi et al. [7].

Indeed, studying their data in detail, Luppi et al. [7] showed some surprising results: First, a relatively high percentage of unstimulated monocytes produce IL-12 [mean percentage of IL-12-producing cells at the end of pregnancy, 33% (Fig. 5 in their paper)], and in an individual case, 71% of circulating monocytes are producing IL-12 (Fig. 4 of their paper). Similar results were found for tumor necrosis factor α (TNF- α ; ~20% of circulating monocytes during pregnancy produce TNF- α , and in an individual case, up to 43% of circulating monocytes produce TNF- α). Only when male volunteers were injected with endotoxin did we observe cytokine production in unstimulated cells [8]; however, the percentage of cytokine-producing cells was never more than 10%, and even these low percentages of circulating, cytokine-producing cells were pyrogenic in these males. Spontaneous production of IL-12 and TNF- α during normal pregnancy is a very dangerous situation, especially in light of the well-known, harmful effects of type 1 cytokines, such as IL-12 and TNF- α , for the fetus [9]. Therefore, from a biological point of view, it is not very likely that during normal pregnancy, 20–30% of the monocytes are spontaneously producing IL-12 and/or TNF- α .

Indeed, we [4] and others [3, 5] have also looked at monocyte intracellular cytokine production during third-trimester pregnancy or immediately after pregnancy and did not find significant percentages of monocytes spontaneously producing

TNF- α or IL-12 (i.e., percentages were below 5%). Additionally, we have measured intracellular monocyte cytokine production (TNF- α , IL-12, or IL-1 β) in various reproductive phases in humans and have never observed production of cytokines in unstimulated cells [8, 10]. This prompted us to question the methodology used by Luppi et al. [7].

The other surprising finding in the paper by Luppi et al. [7] was the fact that IL-12 production decreased after endotoxin or phorbol 12-myristate 13-acetate/ionomycin stimulation of monocytes of third-trimester women. This is in sharp contrast to all other papers studying endotoxin-induced IL-12 production, as it is common knowledge that endotoxin is a stimulator of IL-12 production [11]. Endotoxin not only stimulates monocyte IL-12 production in nonpregnant individuals or in males [8] but also during pregnancy [3–5].

A remarkable finding concerning the methodology of the present paper was the use of the isotype controls for analysis of intracellular cytokine production. Isotype controls are classically used to set the lower limits for staining positivity, such as done by Luppi et al. [7]. There has, however, been discussion as to the limitations of the use of isotype controls in flow cytometry [5, 12]. Issues to be considered are the fact that antibodies, including isotype controls, are currently produced by hybridomas. They will structurally differ from each other, even within the same immunoglobulin (Ig) isotype and may therefore have different binding properties [5, 12]. Second, different companies use different protocols to produce, purify, and chemically conjugate antibodies with fluorochromes. Therefore, the fluorochrome-to-antibody ratio may differ among different companies [5, 12].

This indicates that if we use isotype controls, the choice of the isotype control should be made on the basis of an identical Ig subclass from the same species, and it is also very important to purchase isotype controls from the same company as the antibody itself [5, 12]. Unfortunately, Luppi et al. [7] did not meet all above-mentioned criteria. This holds, for instance, for anti-IL-12 and its isotype control, which were purchased from different companies. We question, therefore, whether differences in results between the paper of Luppi et al. [7] and the other papers on unstimulated and stimulated IL-12 production by monocytes [3, 5] may be a result of use of an inappropriate isotype control by Luppi et al. [7]. Possibly, this may also hold

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for other differences in results, for instance, differences in adhesion molecule expression between this paper of Luppi et al. [7] and other papers [3–6], including their own paper.

REFERENCES

1. Luppi, P., Haluszczak, C., Betters, D., Richard, C. A., Trucco, M., DeLoia, J. A. (2002) Monocytes are progressively activated in the circulation of pregnant women. *J. Leukoc. Biol.* **72**, 874–884.
2. Sacks, G. P., Sargent, I. L., Redman, C. W. G. (1999) An innate view of human pregnancy. *Immunol. Today* **20**, 114–118.
3. Sacks, G. P., Redman, C. W. G., Sargent, I. L. (2003) Monocytes are primed to produce the Th1 type cytokine IL-12 in normal human pregnancy: an intracellular flow cytometric analysis of peripheral blood mononuclear cells. *Clin. Exp. Immunol.* **131**, 490–497.
4. Veenstra van Nieuwenhoven, A. L., Bouman, A., Moes, H., Heineman, M. J., de Leij, F. M. L. H., Santema, J., Faas, M. M. (2003) Endotoxin-induced cytokine production of monocytes of third trimester pregnant women compared to women in the follicular phase of the menstrual cycle. *Am. J. Obstet. Gynecol.* **188**, 1073–1077.
5. Hebra, A., Strange, P., Egbert, J. M., Ali, M., Mullinax, A., Buchanan, E. (2001) Intracellular cytokine production by fetal and adult monocytes. *J. Pediatr. Surg.* **36**, 1321–1326.
6. Sacks, G. P., Studena, K., Sargent, I. L., Redman, C. W. G. (1998) Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am. J. Obstet. Gynecol.* **179**, 80–86.
7. Luppi, P., Haluszczak, C., Trucco, M., DeLoia, J. A. (2002) Normal pregnancy is associated with peripheral leukocyte activation. *Am. J. Reprod. Immunol.* **47**, 72–81.
8. Faas, M. M., Moes, H., Fijen, J. W., Muller Kobold, A. C., Tulleken, J. E., Zijlstra, J. G. (2002) Monocyte intracellular cytokine production during human endotoxaemia with or without a second in vitro LPS challenge: effect of RWJ067657, a p36 MAP-kinase inhibitor, on LPS-hyporesponsiveness. *Clin. Exp. Immunol.* **127**, 337–343.
9. Wegmann, T. G., Lin, H., Guilbert, L., Mosmann, T. R. (1993) Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol. Today* **14**, 353–356.
10. Bouman, A., Moes, H., Heineman, M. J., de Leij, F. M. L. H., Faas, M. M. (2001) The luteal phase of the ovarian cycle: increasing sensitivity of monocytes to endotoxin. *Fertil. Steril.* **76**, 555–559.
11. Snijders, A., Hilken, C. M., van der Pouw Kraan, T. C., Engel, M., Aarden, L. A., Kapsenberg, M. L. (1996) Regulation of bioactive IL-12 production in lipopolysaccharide-stimulated human monocytes is determined by the expression of the p35 subunit. *J. Immunol.* **156**, 1207–1212.
12. Keeney, M., Gratama, J. W., Chin-Yee, I. H., Sutherland, D. R. (1998) Isotype controls in the analysis of lymphocytes and CD34+ stem and progenitor cells by flow cytometry—time to let go! *Cytometry* **34**, 280–283.