

ANALYSIS OF OVARIAN MACROPHAGE POPULATIONS USING MACROPHAGE-SPECIFIC GREEN FLUORESCENT PROTEIN (GFP) TRANSGENIC MICE

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Macrophages represent a major immune cell type in reproductive tissues and are thought to regulate multiple aspects of reproduction, including ovarian function. We have previously shown distinctive phenotypes and functions of ovarian macrophages such that many immunological mediators, such as cytokines and hormone receptors, are uniquely regulated within these cells across the oestrus cycle. In order to isolate macrophages from ovarian tissue by fluorescence activated cell sorting (FACS), we acquired transgenic mice (from DA Hume, Institute for Molecular Bioscience, University of Queensland) which express GFP exclusively in macrophages (1). In these mice GFP is expressed under direction of the *c-fms* gene promoter, which encodes the receptor for colony-stimulating factor-1 (CSF-1R), a major macrophage growth factor. Using flow cytometry we confirmed that 95% of peritoneal macrophages express GFP and 88% co-express GFP and the classical macrophage marker F4/80. The distribution of GFP+ macrophages in tissues was co-localized with macrophage markers F4/80 and major histocompatibility complex class II (MHCII) by immunohistochemistry using phycoerythrin (PE)-labelled antibodies. The liver, uterus and oviduct exhibited many GFP+ cells in characteristic macrophage distributions. Furthermore, GFP fluorescence was tightly co-localized with PE fluorescence of either F4/80 or MHCII, indicating that CSF-1R is expressed in the macrophages of these tissues. In contrast, macrophages in the ovary were positive for F4/80 and MHCII, but rarely expressed GFP. Thus unlike macrophages of other reproductive tissues, ovarian macrophages do not consistently express CSF-1R. In ovaries from gonadotrophin-primed immature females, GFP was not expressed in macrophages (F4/80+/MHCII+) surrounding follicles but was detected in macrophages within the regressing corpus luteum. Thus CSF-1R is a hormonally regulated gene, expressed only in specific subsets of ovarian macrophages suggesting that CSF-1 controls functional activities of ovarian macrophages at specific stages of the ovarian cycle.

(1) Sasmono, R. T., *et al.* (2003) *Blood* **101**, 1155–1163.