JEM Journal of Experimental Medicine

INSIGHTS

FRC-derived retinoic acid: The key to unlocking milky spots

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Milky spots of the omentum enable lymphocyte access to the peritoneal cavity. In this issue of *JEM*, Yoshihara and Okabe (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20221813) demonstrate how secretion of retinoic acid by fibroblastic reticular cells allows lymphocyte entry into milky spots and the peritoneal cavity.

The omentum is a visceral adipose tissue (VAT) nicknamed the "policeman of the abdomen" due to its ability to migrate to sites of inflammation and organ damage and to confer immune protection in the abdomen. Immune cell clusters termed "milky spots," which develop just below the surface of the omentum, underly this role by providing immune surveillance against peritoneal infection and by maintaining key immune cell types resident in milky spots and the peritoneal cavity. Similar atypical lymphoid structures, called fat associated lymphoid clusters (FALCs), are also found in the mesenteries, mediastinum, and pericardium and support immune function in the peritoneal, pleural, and pericardial cavities (Moro et al., 2010; Bénézech et al., 2015; Jackson-Jones et al., 2016). The role of stromal cells in the recruitment, organization, survival, and function of immune cells in secondary lymphoid organs is well described (Krishnamurty and Turley, 2020). However, the nature of the immune-stromal interactions supporting immune cell functions in milky spots is still poorly understood.

In this paper, Yoshihara and Okabe (2023) first characterized the capacity of omental stromal cells to produce retinoic acid (RA) by analyzing retinol dehydrogenase metabolic activity using flow cytometry. They confirmed that stromal cells of both mesothelial and fibroblastic origin have the capacity to produce RA, as shown previously (Buechler et al., 2019). However, they found that a subtype of milky spot fibroblasts expressing TIE2 had significantly higher retinol dehydrogenase metabolic activity and expressed higher levels of *Aldh1a2* (the rate limiting enzyme in RA synthesis). These cells combined a transcriptional signature characteristic of fibroblast reticular cells (FRCs) such as *Ccl19*, *Col14a1*, *Col14a1*, and *Dpt* with the expression of genes distinguishing them from other FRC subtypes found in milky spots and lymph nodes, such as *Tie2* and *Periostin* (*Postn*). Using mice expressing the diphteria toxin receptor under the *Postn* promoter (*Postn*^{Dtr} mice), *Aldh1a2*$^{+}$ FRCs were selectively depleted by intraperitoneal diphtheria toxin administration, allowing the authors to demonstrate the importance of these cells in the recruitment of circulating T and B2 cells into milky spots. Ablation of *Aldh1a2*$^{+}$ FRCs led to decreased *Cxcl12* expression in the omentum, and *Aldh1a2*$^{+}$ FRCs were shown to be required for CXCL12 expression on the luminal side of high endothelial venules (HEVs), meaning that downregulation of this chemokine is the mechanism behind impaired lymphocyte recruitment in milky spots. Both FRCs and endothelial cells express *Cxcl12*, but exposure to RA only upregulated CXCL12 expression on endothelial cells in vitro, suggesting that this was the mechanism by which *Aldh1a2*$^{+}$ FRCs influence lymphocyte entry. Importantly, they showed that depletion of *Aldh1a2*$^{+}$ FRCs led to loss of T and B2 cells in the peritoneal cavity, stressing the importance of milky spots HEVs as gateway to the peritoneal cavity.

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This work is a significant addition to the burgeoning field investigating the role of stromal cells in the organization and function of milky spots and FALCs (see figure). In secondary lymphoid organs, different stromal cell types express chemoattractants enabling the division of lymphocytes into T and B cell zones, supporting cell survival and regulating the cellular interactions that generate an effective immune response. In contrast, milky spots and FALCs lack distinct zonal organization, as well as other structural features of lymph nodes such as a capsule and subcapsular sinus (Krishnamurty and Turley, 2020). Despite this, it is increasingly clear that immune cell maintenance, interaction,

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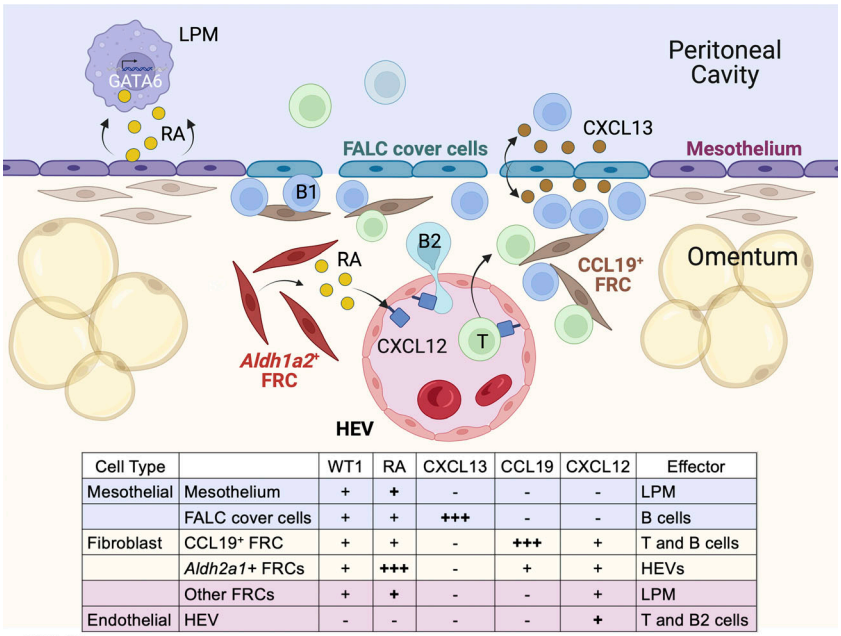

Cell Type		WT1	RA	CXCL13	CCL19	CXCL12	Effector
Mesothelial	Mesothelium	+	+	-	-	-	LPM
	FALC cover cells	+	+	+++	-	-	B cells
Fibroblast	CCL19⁺ FRC	+	+	-	+++	+	T and B cells
	Aldh2a1+ FRCs	+	+++	-	+	+	HEVs
	Other FRCs	+	+	-	-	+	LPM
Endothelial	HEV	-	-	-	-	+	T and B2 cells

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RA production by FRCs is the key to unlock milky spots. *Aldh1a2*⁺ FRCs generate RA that induces the expression and presentation of CXCL12 on the luminal face of HEVs, facilitating the recruitment of T and B2 cells in milky spots. RA generated by WT1⁺ mesothelial cells and other fibroblasts is sufficient to promote the localization and the tissue-specific phenotype of GATA6⁺ LPMs. The surface of milky spots is covered by CXCL13⁺ FALC cover cells, while their internal architecture is supported by CCL19⁺ FRCs. The table summarizes the expression of key factors by stromal cell, with "–" for no expression, "+" for low expression, and "+++" for high expression. Created with Biorender.com.

and migration in VAT and their associated cavities are at least partly regulated by a constellation of stromal cells, including stromal cells of mesothelial origin, which are unique to milky spots and FALCs. The VAT is entirely covered by mesothelial cells and contains fibroblasts, both of which express the transcription factor Wilm's Tumor 1 (WT1) and generate RA, which play a critical role in the maintenance of GATA6⁺ large peritoneal macrophage (LPM) population at homeostasis (Buechler et al., 2019). The cavity surface of FALCs is covered by cells of mesothelial origin (termed FALC cover cells) expressing CXCL13, a chemokine critical for recruitment of B cells into FALCs and milky spots. FALC cover cells express many inflammatory chemokines, including CXCL1, which mediates the recruitment of neutrophils to FALCs and milky spots during peritonitis (Jackson-Jones et al., 2020). The architecture of FALCs is supported by FRCs expressing CCL19, and during peritoneal immune challenge, MYD88-dependent activation of these CCL19⁺ FRCs leads to the recruitment of inflammatory monocytes and supports T cell–dependent B cell activation (Perez-Shibayama et al., 2018). To this list the *Aldh1a2*⁺ FRCs can now be added, along with an understanding of the mechanisms by which T and B2 cells are recruited into the milky spots at homeostasis. However, Yoshihara and Okabe's paper also opens new and interesting questions: What are the mechanisms determining whether lymphocytes remain in the milky spots or exit into the peritoneal cavity? And how is this system altered or overridden during peritonitis and the generation of a local immune response?

This study provides insights into the important role that RA plays in the maintenance of FALCs/milky spots and the immune cells of the serous cavities. RA is a metabolite of vitamin A and has several important immune functions. It is crucial for the development of lymph nodes through induction of the chemokine CXCL13 on stromal organizer cells (Van De Pavert et al., 2009), and to promote class switching of B cells to an IgA isotype (Bos et al., 2022). In the context of the serous cavities, Okabe and Medzhitov demonstrated that RA is the signal that drives the localization and the tissue-specific phenotype of LPMs through induction of the master transcription factor GATA6 (Okabe and Medzhitov, 2014). Buechler et al. (2019) showed that WT1⁺ stromal cells are critical for the maintenance of LPMs and are the source of RA in homeostasis, but could not judge the respective contribution of mesothelial cells and fibroblasts in supporting LPMs. Yoshihara and Okabe (2023) here show that in the omentum a subtype of FRCs expressing high levels of *Aldh1a2*⁺ metabolizes more RA than any other stromal cell type. Intriguingly, they show that *Aldh1a2*⁺ FRCs play no role in supporting LPM populations, as these were not affected by their depletion in *Postn*^{Dtr} mice. Conversely, the remaining RA producing WT1⁺ stromal cells were not sufficient to maintain the expression of CXCL12 in HEVs and to recruit circulating lymphocytes into milky spots. The non-redundant roles played by these cell types, which ostensibly function through the same metabolic pathway, raises the question of how RA produced from distinct cellular sources has different downstream effects. Is it merely a question of co-localization of retinol metabolizing cells close to their effectors? Or are there alternative mechanisms that confer specificity, such as co-stimulatory signals or specialized transport pathways? There are many other unanswered questions around the effect of RA on milky spots. For example, is it required for CXCL13 expression (as it is in lymph nodes), without which B cell migration into milky spots does not occur (Ansel et al., 2002)? Is this only a homeostatic mechanism or is it altered during disease? The insights from this paper that distinct sources of RA can have different immune effects may help to guide further work in this area.

Immune cells of milky spots, FALCs, and their associated serous cavities are dynamic populations, whose function and survival are dependent in part on their movement between anatomical compartments. This is well demonstrated by B1 cells, which exist as a self-sustaining population in the serous cavities but migrate into FALCs to be activated, from which they go to other tissues such as the spleen and bone marrow (Baumgarth, 2013). *Aldh1a2*⁺ FRC induced expression of CXCL12 on HEVs of milky spots is a novel pathway explaining how T and B2 cells populate the serous cavities at homeostasis, driving their recruitment from the circulation. An interesting point from this study is the finding that *Cxcl12* was also found in other omental FRCs, and that its

expression was not dependent on RA. As CXCL12 expression supports ectopic lymphoid follicle formation in other tissues, the authors speculate that RA dependent endothelial CXCL12 may represent a homeostatic control mechanism, and during inflammation FRC CXCL12 may drive lymphoid expansion. Further studies in inflammatory models would be needed to determine whether this is the case, and to understand what stimulates FRC CXCL12 expression in the omentum. A more precise understanding of the pathways regulating immune cell trafficking into VAT could potentially have significant therapeutic benefits. As these tissues are the gateways to the serous cavities, the ability to manipulate these pathways to improve the response to infection, promote tissue regeneration, or limit excessive scarring and adhesion formation could be beneficial in many clinical settings. This may also be significant in obesity, where accumulation of pro-inflammatory T and B2 cells in the VAT helps drive local and systemic inflammation contributing to the pathology of obesity-related disease (Oleinika et al., 2022). In this context, an understanding of what controls T and B2 cell movement into fat depots could provide the basis for future work looking to address obesity-associated fat inflammation.

References

Ansel, K.M., et al. 2002. *Immunity*. https://doi.org/10.1016/S1074-7613(01)00257-6

Baumgarth, N. 2013. *Adv. Exp. Med. Biol.* https://doi.org/10.1007/978-1-4614-6217-0_7

Bénézech, C., et al. 2015. *Nat. Immunol.* https://doi.org/10.1038/ni.3215

Bos, A., et al. 2022. *Mucosal Immunol.* https://doi.org/10.1038/s41385-022-00509-8

Buechler, M.B., et al. 2019. *Immunity*. https://doi.org/10.1016/j.immuni.2019.05.010

Jackson-Jones, L.H., et al. 2016.*Nat. Commun.* https://doi.org/10.1038/ncomms12651

Jackson-Jones, L.H., et al. 2020. *Immunity*. https://doi.org/10.1016/j.immuni.2020.03.011

Krishnamurty, A.T., and S.J. Turley. 2020. *Nat. Immunol.* https://doi.org/10.1038/s41590-020-0635-3

Moro, K., et al. 2010. *Nature*. https://doi.org/10.1038/nature08636

Okabe, Y., and R. Medzhitov. 2014. *Cell*. https://doi.org/10.1016/j.cell.2014.04.016

Oleinika, K., et al. 2022. *Clin. Exp. Immunol.* https://doi.org/10.1093/cei/uxac079

van de Pavert, S.A., et al. 2009. *Nat. Immunol.* https://doi.org/10.1038/ni.1789

Perez-Shibayama, C., et al. 2018. *Sci. Immunol.* https://doi.org/10.1126/sciimmunol.aar4539

Yoshihara, T., and Y. Okabe. 2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20221813