M1 macrophages, key contributors to lymphoid neogenesis in atherosclerotic aorta

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This editorial refers to ‘M1 macrophages act as LTβR-independent lymphoid tissue inducer cells during atherosclerosis-related lymphoid neogenesis’ by K. Guedj et al., pp. 434–443, this issue.

The immune system has evolved to optimize the chances of encounter between rare antigen-specific T and B cells of the adaptive immune system with antigen presenting cells of the innate immune system through development of organized secondary lymphoid organs (SLOs) such as the spleen and lymph nodes. This system efficiently eradicates pathogens, including foreign antigens. However, when antigens are constantly replenished, as in atherosclerosis, this can lead to local or systemic chronic inflammation. Under such conditions, tissues that persistently harbour target antigens are infiltrated by cellular effectors of the immune system, including T cells, B cells, and macrophages as well as dendritic cells and plasma cells. These cellular elements frequently organize themselves anatomically and functionally similar to SLOs, leading to de novo formation of B-cell foci and T-cell areas by a process called lymphoid neogenesis or tertiary lymphoid organ (TLO) formation. TLOs have been observed in autoimmune diseases such as autoimmune thyroiditis, rheumatoid arthritis, and myasthenia gravis as well as in chronic allograft rejection,1,2 diabetes,3 and in atherosclerosis.4 Whilst the role of TLOs is still yet to be fully elucidated in diseases such as atherosclerosis, there is evidence that like SLOs, they can contribute to immune disease progression.5 For example, B cells in TLOs within lungs of patients with rheumatoid arthritis produce rheumatoid factor and antibodies against citrullinated proteins that are found in patients with this disease.6 Also, disruption of TLOs in a model of autoimmune diabetes prevents formation of autoaggressive T cells and progression of diabetes,7 and their disruption in salivary glands in a model of Sjögren’s disease leads to partial restoration of salivary function.8

Formation of TLOs at sites of chronic inflammation follows many of the pathways involved in secondary lymphoid organogenesis occurring before birth. During SLO development CD3-CD4⁺CD31⁺ROD⁺IL-7R⁺CD45⁺LTi (lymphoid tissue inducer) cells express lymphotoxin (LT)αβ2 and upon interaction with stromal lymphoid tissue organizer (LTo) cells trigger lymphotoxin β receptor (LTβR) signalling resulting in increased expression of adhesion molecules such as vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), mucosal addressin cell adhesion molecule-1 (MADCAM-1) and chemokines CC ligand (CCL)19 and CCL21, attracting T and dendritic cells and chemokine CXC ligand (CXCL)13 attracting B cells. In addition, production of lymphangiogenic growth factors leads to formation of Lyve-1⁺ lymphatic vessels.9,10 During tertiary lymphoid organogenesis in the adventitia of atherosclerotic abdominal aorta, medial smooth muscle takes the place of LTos. Similar to LTos, medial smooth muscle cells underlying the lesion are thought to be activated by LRβR signalling to express key chemokines such as CXCL13 and CCL21 which are crucial for TLO development11 but the origin of cells responsible for their activation has eluded identification. Guedj et al. demonstrate that M1 macrophages act in a manner similar to LTi cells to stimulate development of TLOs associated with atherosclerotic lesions (Figure 1).12 Like the association of TLOs with age and advanced atherosclerotic lesions,13 M1 macrophages are also most abundant in aged ApoE⁻/⁻ mice with advanced lesions13 and are associated with high expression of TNF-α and lymphotoxin-α (LT-α). They firmly establish the importance of M1 macrophages in TLO formation by demonstrating that vascular smooth muscle cells activated by conditioned media from M1 macrophages and implanted subcutaneously into mice in Matrigel result in TLO-like structures containing T and B cells. They also demonstrate that TNF-α and LT-α as well as conditioned medium from macrophages stimulate vascular smooth muscle cells to secrete chemokines CXCL16, CCL19 and CCL20. Previous in vitro studies also using cultured mouse medial aortic smooth muscle cells indicate that combined TNFR-1 and LTβR signalling induces smooth muscle cells to differentiate into LT-like cells which express a pattern of chemokines required for TLO formation.14 Surprisingly, they find that blockade of TNFR1 and TNFR2 but not LTβR attenuated their secretion. Rather than using LTβR-lg to antagonize LT-α, they perform orthotopic transplantation of aorta from LTβR⁻/⁻ mice into ApoE⁻/⁻ mice to confirm their in vitro findings, demonstrating that transplanted aortic segments from LTβR⁻/⁻ mice still develop adventitial lymphoid structures containing T- and B-cell-rich areas together with a LYVE-1⁺ lymphatic system associated with atherosclerotic lesions. To further support a major role for TNF-α, they demonstrate that neutralizing TNF-α decreases germinal centre B cells and adventitial TLOs. Unfortunately they did not elucidate the cellular source of TNF-α responsible for these effects. While macrophages are major producers of TNF-α, B-cell-derived TNF-α, both membrane-bound and soluble forms, also play a key role in germinal
centre formation with T-cell-derived TNF-α providing a complementary signal.15

The most important findings of the present investigation is the identifi-
cation of M1 macrophages as the cells responsible for activating
medial vascular smooth muscle cells to secrete chemokines that lead
to adventitial TLOs associated with advanced atherosclerotic lesions.
These appear to be macrophages within atherosclerotic lesions secre-
ting TNF-α and LT-α. Whilst a role for LTβR receptor signalling is defini-
tively excluded, it is likely that both TNFα and LT-α participate. LT is
expressed in either membrane-bound or secreted forms; the secreted
form (LT-α) binds both TNFR1 and TNFR2 with high affinity, receptors
the authors have implicated in TLO formation. In contrast the trans-
membrane heterotrimetric form (one LT-α plus two LT-β) selectively
binds to LTβR with high affinity;16 TNF-α also binds to TNFR1 and
TNFR2 but not LTβR. Earlier studies demonstrating that LTβR-Ig sup-
press TLOs in atherosclerotic ApoE−/− mice do not exclude a role
for TNFR1/TNFR2 signalling11 rather they demonstrate roles for LT.
Whether LTβR-Ig treatment has effects on TNF-α expression similar
to LT-α gene deletion remains to be determined.

While the current study has significantly advanced our understanding
of mechanisms involved in development of TLOs in atherosclerotic
aorta, important questions remain. It is still unclear how cytokines
and chemokines produced by cells within TLOs—dendritic cells, T and B
cells contribute to organization of TLOs; surface lymphotixin
expressed by B cells is critical for organization of SLOs. Also, do interac-
tions between mature CD4+ T cells with dendritic cells contribute to
adventitial TLO development? The present study provides no insight
as to why TLOs are largely restricted to abdominal aorta; is this
related to aortic media thickness or other properties of the aorta?
Finally, the identification of M1 macrophages as LTI-like cells is a major
step in our ability to specifically modulate TLO development and
ultimately, gain novel insights into the pathophysiological significance
of TLOs in advanced atherosclerotic lesions.

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References
1. Drayton DL, Liao S, Mounzer RH, Ruddle NH. Lymphoid organ development: from on-
3. Kendall PL, Yu G, Woodward EJ, Thomas JW. Tertiary lymphoid structures in the pan-
creas promote selection of B lymphocytes in autoimmune diabetes. J Immunol 2007;178:
5643—5651.
4. Houthuijs MA, de Boer OJ, van der Loos CM, van der Wal AC, Becker AE. Adventitial
infiltrates associated with advanced atherosclerotic plaques: structural organization sug-
gests generation of local humoral immune responses. J Pathol 2006;206:178
6. Rangel-Moreno J, Hartson L, Navarro C, Gaxiola M, Selman M, Randall TD. Inducible
broncho-associated lymphoid tissue (iBALT) in patients with pulmonary complications
vation of naive T cells in the islets by lymphotixin beta receptor-dependent tertiary
8. Guturu MK, Skarstein K, Papandile A, Browning JL, Fava RA, Bolstad AI. Blockade of
lymphotixin-beta receptor signaling reduces aspects of Sjogren’s syndrome in salivary
9. Vonderhoff MF, Greuter M, Gavese G, Eweaut D, Dewint P, Ware CF et al. LTβR sig-
naling induces cytokine expression and up-regulates lymphangiogenic factors in lymph
11. Grabner P, Lotzer K, Dopping S, Hildner M, Radke D, Beer M et al. Lymphotixin β re-
ceptor signaling promotes tertiary lymphoid organogenesis in the aorta adventitia of age


