Macrophages form a protective cellular barrier in joints

Buckley, Christopher

DOI:
10.1038/d41586-019-02340-x
10.1038/d41586-019-02340-x.

Document Version
Peer reviewed version

Citation for published version (Harvard):
Buckley, C 2019, 'Macrophages form a protective cellular barrier in joints', *Nature*, vol. 572, no. 7771, pp. 590-592. https://doi.org/10.1038/d41586-019-02340-x, https://doi.org/10.1038/d41586-019-02340-x.

https://doi.org/10.1038/d41586-019-02340-x

ARTHRITIS[OK?]

An immune-cell barrier protects joints

Inflammation and the repair of damaged tissues are regulated by immune cells called macrophages. The finding that they form a layer that shields mouse joints from damage has implications for the treatment of arthritis.

CHRISTOPHER D. BUCKLEY

Immune cells called macrophages commonly function as scavenger-like (phagocytic) cells that ingest and remove damaged cells. Writing in *Nature*, Culemann *et al.*[1] report that the macrophages present in joints also fulfil an unexpectedly different role.

Macrophages derive from two main cellular lineages[2]. One lineage arises from bone-marrow-derived immune cells called monocytes. The other lineage is monocyte independent, and is derived from cells that disperse into the tissues during embryonic development[2]. The tissue-resident macrophages in this lineage have distinctive gene-expression profiles[3,4] that depend on the particular tissue in which they reside.

Rheumatoid arthritis is an ~~autoimmune~~ disease associated with inflammation and the destruction of the cartilage and bone in joints, and macrophages have a key role in the initiation of this condition. However, little is known about the relative contribution of the two lineages of macrophages to the development and function of joints in health and disease. To add to the complexity, macrophages exist as various subsets, some of which are pro-inflammatory whereas others limit inflammation and aid tissue repair[5].

To study macrophages, the authors began by focusing on a protein called CX3CR1, which is expressed on monocytes and macrophages. The authors engineered CX3CR1-expressing cells in mice to make a red fluorescent protein so that the cells could be tracked *in vivo*. These cells were monitored in knee joints using an approach called 3D light-sheet fluorescence microscopy, and the joint tissue was treated using a technique that enabled the authors to obtain 'optical clearance', which improves the visualization of internal structures[6].

The authors' observations unexpectedly revealed that CX3CR1-expressing macrophages exist as a layer of cells that forms a barrier, similar to a thin protective membrane, in the healthy joint (Fig. 1). This barrier forms as an outer layer of cells in the synovium, a region of the tissue that lines the joint. The barrier layer forms in a part of the synovium ~~called~~ called the lining layer, and it physically separates the synovial fluid (which bathes the joint) from the sublining layers of the synovium. The CX3CR1-expressing barrier-forming macrophages are found adjacent to a layer of cells called fibroblasts in the lining layer.

The authors carried out RNA sequencing, including single-cell sequencing, to profile the barrier macrophages. These cells express genes typically associated with barrier formation in a type of non-immune cell called an epithelial cell. For example, the macrophage profile included genes that encode proteins associated with the formation of a structure called a tight junction that connects epithelial cells by forming a 'seal' between adjacent epithelial cells. This is surprising, because macrophages are usually thought of as having a signalling or scavenging role, rather than having a structural, barrier-like function.

Using a mouse model of arthritis in which macrophages could be tracked by engineering them to be fluorescent, the authors observed that the barrier layer was highly dynamic. When arthritis was induced, the layer underwent active remodelling that loosened the physical interactions between barrier macrophages and lining-layer fibroblasts. Like other types of tissue-resident macrophage, the barrier macrophages can ingest and remove inflammatory immune cells called neutrophils that have accumulated and died in the synovial fluid during arthritis.

When the authors induced arthritis in mice at the same time as they disrupted the barrier-forming layer of macrophages through genetic or pharmacological manipulation, arthritis was exacerbated compared with animals in which the layer was intact. It would be interesting to test whether transferring barrier macrophages directly into mouse joints could suppress arthritis.

To explore the origin of the barrier-forming, CX3CR1-expressing macrophages, the authors used intricate fate-mapping experiments, which revealed that these cells are not derived from monocytes. They also found that monocytes did not give rise to the other type of macrophage that resides in the joint, termed an

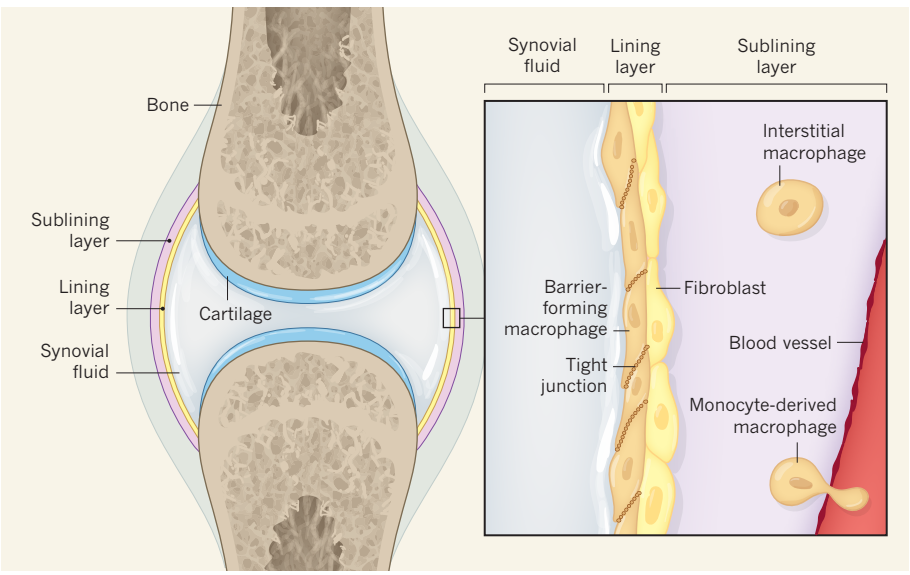

Figure 1 | Barrier macrophages in the joint. Culemann *et al.*[1] studied immune cells called macrophages in mouse and human joints. Joints are surrounded by a tissue called the synovium, which is formed from layers of cells called the lining and the sublining layers. The authors discovered that certain macrophages form a cell layer that protects joints from the inflammatory immune-cell attacks on bone and cartilage that are associated with arthritis. This barrier is formed in the lining layer, adjacent to a layer of cells called fibroblasts. The barrier-forming macrophages express proteins associated with a type of barrier-forming cell called an epithelial cell, and these proteins form structures called tight junctions that 'seal' cells together. Barrier-forming macrophages arise from a type of macrophage called an interstitial macrophage, which resides in the sublining layer. By contrast, non-resident macrophages enter the joint from blood vessels. These cells, which can drive inflammation, arise from immune cells called monocytes.

interstitial synovial macrophage, which populates the sublining layer. The authors' data are consistent with a model in which interstitial macrophages give rise to barrier macrophages.

RNA-sequencing experiments revealed that interstitial macrophages can be divided into two groups. One group expressed the gene *Retnla*, whereas the other had a high level of expression of the genes that encode the proteins MHC class II and aquaporin. The macrophages that express MHC class II and aquaporin divide and differentiate either to form barrier macrophages or to form interstitial macrophages that express *Retnla*.

To analyse the macrophage subsets that arise as arthritis develops, compared with those present in an uninflamed joint, the authors carried out further single-cell RNA sequencing. As expected from previous work[7], monocyte-derived macrophages that produce pro-inflammatory molecules accumulated in the arthritic joint. They are recruited into the joint from the bloodstream by exiting blood vessels to enter the sublining layer. During the influx of these pro-inflammatory macrophages, the barrier macrophages maintained their anti-inflammatory role, expressing the proteins needed for them to remove dead neutrophils from the joint.

When the authors compared their single-cell RNA data from mice with similar data sets[8] available from an analysis of the joints of people with rheumatoid arthritis, the gene-expression profiles of the macrophage subsets matched up across the two species. This suggests that cells similar to the barrier and interstitial macrophages in mice might also exist in humans, and thus possibly be relevant to human disease.

The authors found that barrier macrophages were almost totally absent in synovial samples from people with active***[OK for where 'active' should be placed?]*** rheumatoid arthritis, whereas they made up 10% of the macrophage population in samples from people who have osteoarthritis, a type of arthritis that is not associated with inflammation. It would be interesting to learn whether the population of barrier macrophages is restored in people whose rheumatoid arthritis is being successfully treated and in remission.

Culemann and colleagues' work adds to studies[3,4,9] showing that macrophages are exquisitely adapted to the functions they perform in the tissues in which they reside. Barrier macrophages join a growing list of types of macrophage that shield tissues from damage caused by infection, inflammation or cancer. Tissue-resident macrophages can prevent neutrophil-mediated inflammatory damage by physically shielding damaged tissue from neutrophils[10]. Furthermore, in large body cavities, such as those surrounding the gut, heart and lungs, specialized macrophages have been described that are thought to repair mechanical damage[3,9]. These findings also complement the discovery of distinct subsets of fibroblasts, located in the sublining or lining regions of the joint, which, respectively, drive either inflammation or bone damage in arthritis[11]. The challenge that lies ahead will be to develop ways of specifically targeting subsets of macrophages and fibroblasts with the ultimate goal of developing new treatments for people with arthritis. ■

Christopher D. Buckley *is at the Institute for Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, Queen Elizabeth Hospital, Birmingham B15 2WD, UK, and at the Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK.*
e-mail: c.d.buckley@bham.ac.uk

1. Culemann, S. *et al. Nature* https://doi.org/10.1038/s41586-019-1471-1 (2019).
2. Davies, L. C, Jenkins, S. J., Allen, J. E. & Taylor, P. R. *Nature Immunol.* **14**, 986–995 (2013).
3. Buechler, M. B. *et al. Immunity* **51**, 119–130 (2019).
4. Lavin, Y. *et al. Cell* **159**, 1312–1326 (2014).
5. Udalova, I. A., Mantovani, A. & Feldmann, M. *Nature Rev. Rheumatol.* **12**, 472–485 (2016).
6. Tainaka, K., Kuno, A., Kubota, S. I., Murakami, T. & Ueda, H. R. *Annu. Rev. Cell Dev. Biol.* **32**, 713–741 (2016).
7. Misharin, A. V. *et al. Cell Rep.* **9**, 591–604 (2014).
8. Zhang, F. *et al. Nature Immunol.* **20**, 928–942 (2019).
9. Wang, J. & Kubes, P. *Cell* **165**, 668–678 (2016).
10. Uderhardt, S., Martins, A. J., Tsang, J. S., Lammermann, T. & Germain, R. N. *Cell* **177**, 541–555.e517 (2019).
11. Croft, A. P. *et al. Nature* **570**, 246–251 (2019).

Online heading: Macrophages form a protective ~~cell~~*[ular]* barrier in joints

~~**Macrophages form a cell[*ular*] barrier that protects joints against inflammation**~~