
Role of Macrophage-Like Synovial Lining Cells in Localization and Expression of Experimental Arthritis

P.L.E.M. van Lent, A.E.M. Holthuysen, L. van den Bersselaar, N. van Rooijen*, L.B.A. van de Putte, and W.B. van den Berg

Department of Rheumatology, University Hospital St.Radboud, Nijmegen, *Department of Histology, Free University, Amsterdam. The Netherlands.

Synovial Phagocytic Lining Cells: Focus of Inflammation?

Rheumatoid arthritis (RA) is a systemic inflammatory disease predominantly localized in the synovial joints. The reason why synovial joints are the main localization of the inflammatory process is an intriguing and as yet unanswered question. In this paper we focus on the synovial lining layer, a thin layer of cells which covers the surface of the synovial tissue, as a possible starting point of arthritis.

The synovium consists of a sublining layer covered by the lining layer. The sublining layer contains loose connective tissues in which fenestrated bloodvessels are embedded. The one to two cell thick, lining layer is composed of two distinct cell populations (1). The resident type B cells, having characteristics similar to fibroblasts and type A cells which are macrophage-like (1). They are probably descendants from peripheral monocytes (2) and are constantly replaced via the circulation. Under the influence of local factors like granulocyte-macrophage stimulating factor (GM-CSF), monocytes may change in more resident macrophages (3) which have different surface markers.

Prior to the onset of arthritis, phagocytic lining cells become activated by as yet unknown mechanisms. Activation of these cells and other synovial cells may direct the influx of inflammatory cells like polymorphonuclear cells (PMN), monocytes and T cells into the joint tissues, characteristic for arthritis development. In RA, a striking early observation is that increased numbers of cells occupy the synovial lining layer (4). This thickening of the lining layer is mainly due to accretion of macrophages from the periphery, which accumulate around this layer. The contribution of type A cells in the human lining layer increases from approximately 20-30% in health to 80-100% in RA (5). Phagocytic lining cells release many pro-inflammatory factors. Immunolocalization studies have shown that many of these pro-inflammatory mediators are expressed in the lining layer like IL-1α (6), TNFα (7), TGFβ (8), IL-6 (9), IL-8 (10) and GM-CSF (11). The above observations suggest that both in RA and in experimental models, phagocytic lining cells may be involved in attraction of inflammatory cells during onset of arthritis and probably also in arresting these cells within the synovium during the chronic phase. To get further insight in this matter, we carried out several studies in experimental arthritis models.

Selective Depletion of Phagocytic Lining Cells from Murine Knee Joints

There are several ways to study the possible role of phagocytic lining cells in experimental arthritis. One approach may be the isolation of synovial cells from pre-inflammatory synovia and transfer of these cells to control animals. Recent studies by Ramoz-Ruis showed that macrophage-like synovial lining cells isolated from pre-inflammatory synovium from rats with experimental adjuvant arthritis were able to transfer arthritis if these cells were injected into control rats (12). Another approach which was chosen by us, is selective depletion of phagocytic cells prior to arthritis induction or during established arthritis. Several methods to eliminate synovial lining cells have been described. Local deposition of osmium tetroxide (13) or radioisotopes (14) in knee joints indeed showed downregulation of the lining cell function. However, the disadvantages of these methods are that they are nonselective and often cause side effects on other joint tissues (15). In our studies we used liposomes which contain the drug Clodronate (dichloromethylene bisphosphonate: CL2MDP; gift from Boehringer Mannheim). This drug belongs to a class of synthetic compounds structurally related to pyrophosphate, an endogenous regulator of calcium metabolism. We made use of the phagocytic properties of the macrophage which preferentially absorbs the relatively large (1 μM) multi-

Correspondence: P. Van Lent, Lab. Rheumatic/Diseases, University Hospital St. Radboud, Nijmegen, P.O. Box 9101, NL-6500 HB Nijmegen, The Netherlands.
lamellar liposomes. Once inside the cell, the lipid bilayer is disrupted by lysosomal enzymes, the drug sets free and induces cell death probably by its chelating activity (16). Most likely not intracellular Ca$^{2+}$ but Fe$^{3+}$ arrestment leads to macrophage elimination (17). In this way macrophages can be eliminated from various compartments like spleen (18), liver (19), lung (20) and lymph nodes (21) if given via the appropriate routes. We injected clodronate-liposomes directly into the joint compartment. A single injection of six μl liposomes containing 75 μg clodronate into control murine knee joints resulted in selective depletion of phagocytic lining cells. Optimal depletion was found between 6 and 11 days after liposome injection but even after 30 days the lining has not recovered fully (22). No deleterious effects were found on other joint tissues at the time that full depletion of phagocytic lining cells in the knee joint was observed. The free drug, $^{14}$C labeled clodronate is not taken up by cells (23) and had no effect on macrophages. Taken together this simple in vivo macrophage "suicide" technique is selective and shows no deleterious effects on other joint tissues.

**Involvement of Phagocytic Lining Cells in Onset of Experimental Arthritis**

To examine the role of phagocytic lining cells in the onset of arthritis, we studied both a locally and a systemically induced arthritis. The former was induced by cationic immune complexes (ICA). We inject a cationic antigen directly into knee joints of mice which previously were given antibodies directed against the antigen. A severe arthritis then develops characterized by influx of mainly PMNs into the synovial layer (24). Clodronate-liposomes were injected one week prior to arthritis induction. At the time-point of arthritis induction, the lining layer was totally stripped. Arthritis induction in lining cell depleted knee joints caused a significantly reduced influx of PMN (25) (Figure 1). In these experiments, synovitis was scored from haematoxylin/eosin stained total knee joint sections. These data suggest that the phagocytic lining cell is essential in the induction of arthritis in this model.

In the ICA, the arthritis trigger first meets the lining layer. Cationic immune complexes stick predominantly to the lining layer which subsequently becomes activated. These immune complexes have two important properties. First, cationicity (26,27) and size (28) of the immune complexes lead to prolonged persistence in the joint structures. Second, cationic immune complexes induce at least two chemotactic mediators involved in cell influx within this model, i.e. C5a and IL-1. The latter probably acts by inducing IL-8-like chemokines. Production of IL-1 and the chemotactic activity in lining depleted arthritic knee joints were shown to be both significantly decreased (25). Lowering of IL-1 production as a reason for decreased PMN influx was further substantiated by studies showing that neutralization of IL-1 effects by anti-IL-1 antibodies (24) or IL-1 receptor antagonist (29) also decreased PMN influx significantly.

Since lining depletion may only act in the induction phase of a locally induced arthritis in which the trigger meets the lining directly, we further studied the effect of phagocytic lining depletion on the onset and expression of a systemically induced arthritis. As a model we used collagen type II induced arthritis (CIA). DBA/1 lac j mice were immunized with bovine collagen type II in Complete Freund's Adjuvant subcutaneously into the base of the tail. Three weeks later, mice were boosted with 100 μg collagen type II intravenously. One week thereafter bacterial lipopolysaccharide (LPS) was given intraperitoneally to synchronize and elevate the expression of arthritis within the knee joint (30). When no LPS is given, expression of arthritis in the knee joint is usually low and variable. Four days after LPS injection a severe arthritis developed characterized by influx of PMN and monocytes mainly into the synovial layer. In recent studies we injected clodronate-liposomes locally in knee joints at day 9 before the expected expression of arthritis in the knee joint, leading to absence of lining layer at the onset of arthritis. In these experiments we could show that two days after start of arthritis, cell influx into the synovial layer of lining depleted knee joints was significantly decreased (Figure 1). This indicates that phagocytic lining cells are important in the local expression of the systemically induced CIA. Like in the immune complex mediated model, IL-1 seems the dominant cytokine involved in the onset of CIA. Neutralization of IL-1 effects by anti-IL-1 antibodies (31) or IL-1 receptor antagonist (data not shown) fully blocked the influx of cells into the joint cavity. We are currently determining IL-1 levels and chemotactic activity in lining depleted knee joints in CIA.

In CIA both immune complexes and T cells have been described to be important in the onset of arthritis. Like in immune complex mediated arthritis, collagen type II-immune complexes may trigger the lining first. If the T cell is the first drive, this
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cell may be immobilized in the sublining layer. Activated T cells which are mostly found adjacent to macrophages (32), may release cytokines which activate the lining from a distance. The results from lining depletion in both experimental models suggest that the larger part of the chemotactic activity is generated by phagocytic lining cells. Earlier studies revealed that local injection of recombinant murine IL-1 in murine knee joints induced massive influx of PMN (33). In lining depleted knee joint, IL-1 was however not able to induce influx of cells (25), again indicating that PMN chemotactic factors are primarily formed by the lining layer. It therefore appears that IL-1 and probably also TNF, the major products of activated macrophages, might be highly inflammatory by stimulating the release of chemokines by phagocytic lining cells. They produce both \( \alpha \) and \( \beta \) chemokines. The \( \alpha \) chemokines like GRO\( \alpha \) and IL-8 attract PMN, although GRO\( \alpha \) is 10 times more potent (34). In RA synovial fluid chemotactic activity for PMN was inhibited 40 \( \pm \) 5\% upon incubation with neutralizing polyclonal antibody to IL-8 (35). Furthermore phagocytic lining cells release \( \beta \) monokines like chemotactic and activating factor (MCAF) or MCP-1 which predominantly attracts monocytes both in vitro and in vivo (36). MCP-1 also attracts B cells, cytotoxic T cells and CD4+ T cells. Macrophages from RA synovium constitutively express MCP-1 mRNA and protein (37).

So in summary, the phagocytic lining layer seems involved in the onset and expression of

Figure 1: Effect of elimination of phagocytic lining cells from the murine knee joint on the onset of a local (immune complex mediated arthritis: ICA) and a systemically induced (collagen type II induced arthritis: CIA) arthritis. Note the significantly reduced synovitis in the lining depleted clodronate-liposome treated arthritic knee joints. Synovitis was scored in an arbitrary way (0 = no; 1 = minor; 2 = moderate; 3 = marked).
both locally and systemically induced experimental arthritis. The next question was whether the phagocytic lining layer is also involved in the perpetuation of synovitis in an established arthritis. If so, local injection of clodronate-liposomes into large inflamed RA joints might be a possible treatment.

**Involvement of Phagocytic Lining Cells in The Propagation of Established Arthritis**

To investigate this, we used the antigen (mBSA) induced arthritis model (AIA). AIA is induced by injecting a cationic antigen into knee joints of mice which previously are immunized with the antigen in Complete Freund's Adjuvant. A severe acute arthritis is followed by a T cell driven chronic phase (27). The chronic phase is characterized by a synovitis which consists of monocytes and T cells which have been infiltrated from the blood compartment (38). The smouldering synovitis in the knee joint can be reactivated by giving the antigen systemically (39,40). In the chronic phase of this arthritis, the activated lining cells which release chemotactic factors may be responsible for the propagation of the synovitis. To investigate this we injected clodronate-liposomes both in the acute and chronic phase of antigen-induced inflamed knee joints. As controls, PBS or PBS-containing liposomes were given. If clodronate-liposomes were given shortly (6 hrs, 1 day and 3 days) after arthritis onset, no effect was found on synovitis (41). The reason for this was that liposomes are rapidly degraded in an PMN rich inflammatory exudate and fail to reach the lining layer (41). However when clodronate-liposomes were given in the more chronic phase of arthritis (7 days after arthritis onset), we observed a significant downregulation of synovitis at day 21 after arthritis onset (Figure 2). Since most PMNs have disappeared from the joint cavity at this time point, the injected liposomes can easily reach the lining layer. Clodronate-liposomes stained by a fluorescent dye were shown to penetrate deeply into the thickened layer. By eliminating part of the activated phagocytic synovial cells in the lining layer, the synovitis may come to rest. In our hands a single injection of clodronate-liposomes was sufficient to eliminate the synovitis and no relapse was found three or four weeks after liposome treatment (41).

Which mechanisms are involved in persistence of the synovitis are still unknown. The cationic proteins which are used in this model are able to persist in collagenous and cartilagenous joint tissues. Cationic antigens disappear from the synovium much faster than from cartilagenous layers. The cartilage matrix forms a depot of negatively charged proteoglycans, in which large amounts of antigen can persist for prolonged periods forming an antigen reservoir (42,43). Continuous release of small amounts of the antigen from the cartilage may keep the phagocytic lining cells in a permanent state of activation. As cationic antigens are potent inducers of IL-1, they may trigger indirectly the generation of chemokines like IL-8 or MCAF from the lining layer thus sustaining inflammation. That IL-1 might be involved in sustaining inflammation in antigen-induced arthritis was substantiated by the observation that neutralization of IL-1 by anti-IL-1 antibodies decreased the influx of inflammatory cells in later phases of antigen-induced arthritis (44).

**Phagocytic Lining Cells Are Involved in Exacerbation of Smouldering Arthritis**

Apart from sustaining synovitis, the phagocytic lining cells may also be involved in flares of inflammation which can be elicited by giving the antigen either orally (40) or intravenously (41). Small amounts of the antigen reaching the hyperreactive joint can elicit a renewed flare. In the flare reaction, T cells and antigen presenting cells play a dominant role. The flare could be blocked by giving either anti lymphocyte serum (45) or anti-la antibodies (46). Within a couple of hours after induction of the flare, large amounts of IL-1 and IL-6 are generated. Numerous cells, mainly PMNs, infiltrate the synovial layer. Injecting small amounts of mBSA into lining depleted joints showed reduced cell influx (50%) (Figure 2). This was again correlated to reduced levels of IL-1 (data not shown).

Apart from releasing pro-inflammatory molecules, macrophages also release anti-inflammatory molecules. For instance inhibitors of pro-inflammatory cytokines like IL-1 receptor antagonist (IL-1ra) and anti-inflammatory cytokines like IL-4 and IL-10. This negative feedback may thus disappear after lining depletion. Many cells producing anti-inflammatory factors seem however to be resided in the sublining layer which is not reached by the liposomes. IL-1ra which blocks competitively the binding of IL-1 and to their receptors (47), was primarily located in the perivascular regions enriched for macrophages in the sublining layer in 11 of 12 rheumatoid synovial tissues examined (48). T helper 2 cells, producers
of IL-4 and IL-10 also reside in the sublining layer and are closely associated with activated macrophages (49). Thus removal of the superficial lining cells at least leaves a substantial number of cells, located in the sublining tissue, generating anti-inflammatory factors.

In above experimental studies a clear ameliorating effect of lining depletion either on onset or persistence of inflammation in the inflamed knee joint was found. Whether macrophage depletion has beneficial effects of inflammation in the human joint has to be studied. Recent prospective studies in the RA joint, have examined the impact of synovial lining cellularity on the disease course in RA. In milder forms of RA there was less intense accumulation of cells in the synovial lining and little clinical deterioration over a 3-year period of follow up (49). In a second study of radiological progression in RA the number of macrophages but not of lymphocyte populations infiltrating the synovial membrane correlated with deterioration over a 1-year period of follow-up (50).

These studies indicate that eliminating activated phagocytic synovial cells by local injection of clodronate-liposomes might be a promising tool to downregulate inflammation.

References


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**Figure 2:** Effect of phagocytic lining cell depletion from an established arthritic murine knee joint on elongation and exacerbation of chronic synovitis. Note the diminished synovitis if clodronate-liposomes were given during chronic synovitis. Exacerbation of arthritis by giving antigens systemically was also lower if compared to non-treated arthritic knee joints. Synovitis was scored in an arbitrary way (0 = no; 1 = minor; 2 = moderate; 3 = marked).


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