Role of reactive oxygen species in rheumatoid arthritis synovial T lymphocytes

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Chapter 7
Summary and General discussion

In rheumatoid arthritis (RA), an inflammatory infiltrate accumulates and persists in the synovial membrane. The pathophysiological events that initiate and perpetuate the inflammation have still not been elucidated, although it is generally believed that multiple immulogical and genetic factors play a role. Dendritical cells, macrophages, B cells, T cells, synoviocytes, (auto-) antibodies and a wide arrange of cytokines all seem to contribute to the pathogenic events in RA. Starting from the concept of autoimmunity, many still believe T cells play a key role in the pathogenesis of RA. Synovial T cells however, display a number of particular characteristics. While displaying markers of recent activation, synovial T lymphocytes respond poorly to mitogenic stimuli and their cytokine production appears to be suppressed both in situ and in vitro. One of the critical hallmarks from synovial T cells is that they suffer from oxidative stress, as demonstrated by decreased levels of the intracellular anti-oxidant glutathione (GSH) (1). Recently it was shown that the intracellular redox disturbance has critical implications on proximal and distal TCR signaling events. Oxidative stress in synovial fluid T lymphocytes inhibits T cell receptor (TCR)-dependent phosphorylation of pivotal signaling molecules, required for efficient T cell proliferation, and contributes to severe hyporesponsiveness of these cells upon antigenic stimulation (2,3).
Chronic exposure of T lymphocytes to free radicals produced by activated phagocytic cells at the site of inflammation has been proposed to be the major cause of deregulated redox homeostasis in RA. To investigate the exact localization of endogenous ROS production in human synovium we adapted a new cytochemical technique developed by Karnovsky, using the 3,3' diaminobenzidine (DAB) probe and manganese. Free radicals directly react with DAB, forming an insoluble DAB polymer which can be visualised by microscopy. In **chapter 2** we demonstrate that the oxidative stress found in synovial T cells is not the result from exogenous sources but originates from (an) intracellular activated oxidase(s). **In chapter 3** we demonstrate that the oxidase generating ROS in SF T cells is controlled by the small GTPases Ras and Rap1. Whereas introduction of constitutive active Ras in the Jurkat T cell line generates intracellular ROS production via a Ral dependent signalling pathway, introduction of constitutive Rap1 inhibits mitogenic and Ras induced ROS production via a PI3-kinase dependent signaling pathway. Conversely inactive Rap1 increases intracellular ROS production. In SF T cells we find constitutively activated Ras and inactive Rap1. We also show that constitutive Ras activation and inhibition of Rap1 activation are not a result from oxidative stress, but the origin of intracellular free radical production, and that introduction of dominant negative Ras in synovial T cells downregulates the excess ROS production. During the last decade it has become increasingly clear that free radicals can serve as critical second messengers in a wide variety of intracellular signaling events (for review: see introduction). The specific signaling function depends on the kinetics, the localisation and the species of the produced ROS. It was shown that H_2O_2 activates the stress MAPKinases p38 and JNK and the pro-inflammatory transcription factor NF-κB(4-6). Although the exact role of the intracellular free radicals in synovial T cells remains illusive, it is tempting to speculate that intracellular free radicals in T lymphocytes contribute to NF-κB-dependent gene transcription, which in turn results in upregulation of pro-inflammatory cell surface markers such as TNF-α and IL-1 receptors. These proteins play critical roles in the activation of synovial macrophages and fibroblast-like synoviocytes, which in turn secrete cytokines and proteases perpetuating inflammation. Importantly, oxidative stress is also found in other pathological conditions besides RA, e.g. CD4 lymphocytes in patients with AIDS and in

ischemia-reperfusion laesions (7,8). It will be interesting to investigate whether similar oxidases that we found in synovial T lymphocytes are also involved in these diseases.
In **chapter 4** we show that in synovial T cells from RA patients Ras can be activated by a variety of cytokines. Rap1 inhibition is induced by direct cell-cell contact of T lymphocytes with antigen presenting cells (APC), and can be prevented by blocking the co-stimulatory T cell receptor CD28 with CTLA-4. These findings underscore the critical role of free radicals in disturbed T cell function in rheumatoid arthritis. One could speculate that increased ROS production is seen after defective (auto-)antigen presentation by APC or it is possible that in RA T cells are only activated through T cell receptor independent pathways, in casu CD28.
Based on the pro-inflammatory properties of free radicals, one might expect anti-oxidants to have anti-arthritic effects. In animal models, high doses of vitamin C, vitamin E and NAC had beneficial effects on rheumatoid inflammation, related to their antioxidative potential (9-11). Other candidate antioxidants have been identified with potential benefit, including selenium, zinc, manganese, niacinamide, bioflavonoids and β carotene. In **chapter 5**, the results of a double-blind placebo-controlled study with a daily nutrient supplement containing antioxidants and the omega-3 fatty acids eicosapentaenoic acid, docosahexaenoic acid and the omega-6 fatty acid gamma-linolenic acid are presented. We did not find superior clinical benefit at the doses tested as compared to placebo. One could postulate that the doses of the antioxidants used were insufficient. However, treatment of RA patient with high dose NAC did not result in significant clinical improvement either. As described in **chapter 6**, 14 patients with active RA were treated with high dose intravenously administered NAC, adopted from the protocol for acetaminophen intoxication. Only minimal clinical improvement was documented. Whereas in vitro treatment of T lymphocytes with NAC restores T cell defects and hyporesponsiveness, no such effects were seen in the patients that received NAC. Interestingly though, in the SF T cells of all RA patients where we found oxidative stress-dependent signaling defects, we also found NF-κB activation, again suggesting a critical role for free radicals as second messenger in the activation of NF-κB.
Many questions about the exact role of upregulated free radical production in synovial T cells remain. Do ROS aggravate inflammation through activation of NF-κB? Or do they have a benificial effect, downregulating T lymphocytes? Do they play a central role in synovial T cell signaling leading to T cell proliferation and differentiation, or are they involved in T cell apoptosis? Or are they an innocent collateral effect of other cellular processes? Are all synovial T cells suffering from oxidative stress, or is there a subgroup of T lymphocytes that do not display upregulation of intracellular free radicals? Answers to such questions remain important, before answers can be given to questions whether we should counter the oxidative stress with anti-oxidants to enhance SF T cell function, or whether we should specifically target synovial T lymphocytes suffering from oxidative stress, and try to eliminate them. Equally interesting is to examine whether in self-limiting arthritis these ROS-loaded T cells also occur. At present, no such T cells were found in any of the reactive arthritis examined nor in synovial tissue of patients with osteoarthritis.