High dose chemotherapy and autologous hematopoietic stem cell transplantation for rheumatoid arthritis

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CHAPTER 1

INTRODUCTION AND AIM OF STUDIES

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Introduction.

Rheumatoid arthritis (RA) is a systemic disease predominantly affecting the synovial joints. As neither etiology nor pathogenesis is precisely known and no specific histological features exist, classification criteria have been agreed for the diagnosis of RA [1]. Major hallmarks of the disease are chronicity and joint destruction. The disease course is, however, variable. While most patients have relapsing remitting disease others experience progressive disease resulting in joint destruction and severe disability or even death. Severity of the disease is not only determined by joint symptoms, but also by systemic and extra-articular symptoms. Epidemiological studies have shown that overall mortality is increased in comparison with healthy individuals of the same age and sex [2]. Estimates of reduced life expectancy vary from 3 to 18 years. Several negative prognostic factors have been identified that predispose to severe progressive disease such as specific HLA class II antigens and the presence of IgM rheumatoid factor [3]. The relentless disease course in severely affected patients with an adverse prognosis regarding morbidity and mortality has prompted efforts to control the disease aggressively with disease modifying antireumatic drugs [4]. Nevertheless, clinical symptoms and parameters of disease activity are not suppressed in all patients, and these patients in particular may be at risk of developing debilitating disease. The pathogenesis of RA is unknown, but according to current views the interaction of specific cytokine-producing CD4 positive Th1 lymphocytes, macrophages and synoviocytes is thought to underlie chronic synovitis [5] (Figure 1). Compelling evidence points to the pivotal role of T cells in the orchestration of the chronic synovitis in RA [6-8].

The adverse prognosis in a subgroup of RA patients and the recognition that immune mechanisms play a notable role in the pathogenesis of the disease, has led to the development of immunoablation with stem cell rescue as an approach to control RA, because the conditioning regimen may delete the relevant autoreactive lymphocyte populations.

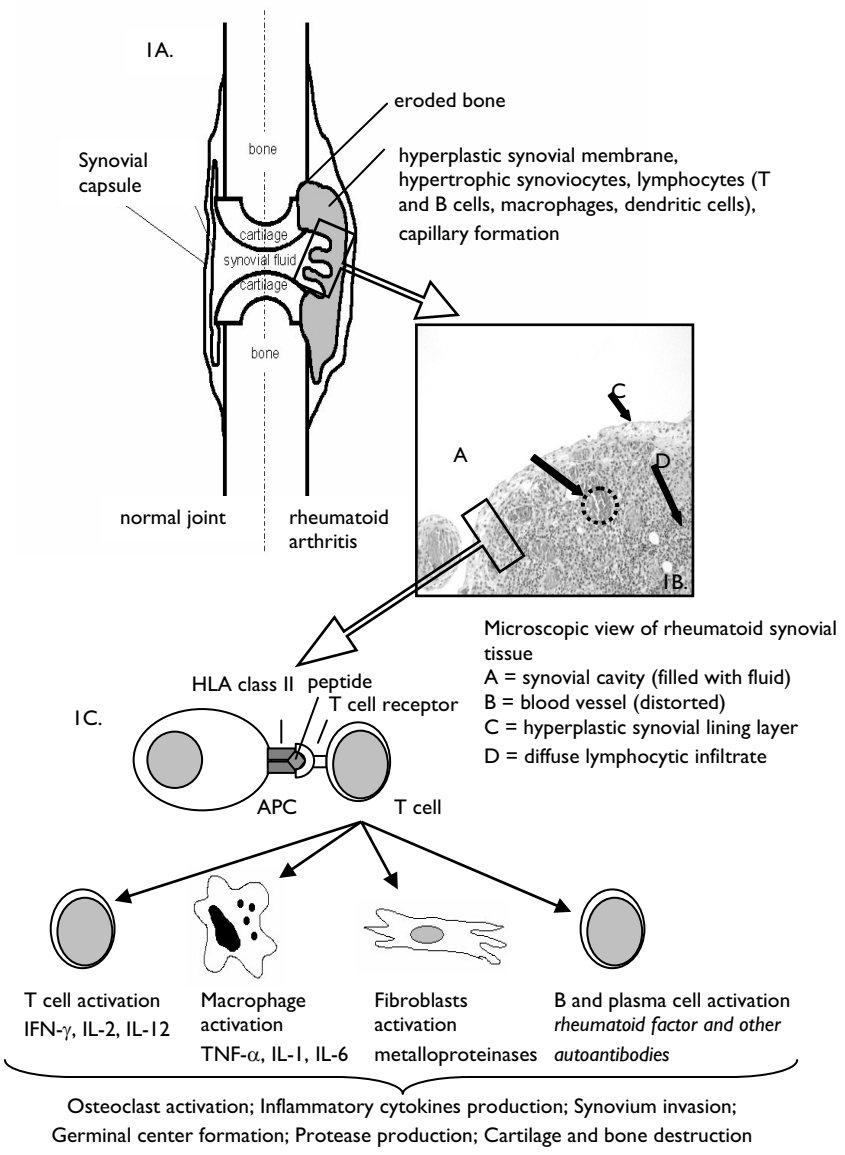

Figure 1. Schematic representation of pathogenetic events in rheumatoid arthritis. 1A. On the left side a normal joint with the synovial membrane (1 to 2 cell layers) and underlying stromal tissue and capsule. Synoviocytes consist of type A (macrophage-like synoviocytes) and type B (fibroblast-like synoviocytes). On the right side a joint of a rheumatoid arthritis patient with a hyperplastic synovial membrane, excessive vascular proliferation and abundant presence of T and B cells, macrophages and dendritic cells. Synovial membrane invades the cartilage and destroys cartilage and bone. 1B. Histology of rheumatoid synovial tissue. 1C. Cells found in the synovial connective tissue and some of their products leading to inflammation and cartilage and bone destruction. APCs (Antigen presenting cells) present peptides in the context of HLA class II molecules to T cells by the T cell receptor. Activation of T cells leads to activation of macrophages, fibroblasts, B cells, plasma cells and proliferation and differentiation of T cells.

Stem cell transplantation in experimental models of autoimmune diseases.
Hematopoietic stem cell transplantation (SCT) as a treatment option in autoimmune diseases was first evaluated in experimental animal models. It was demonstrated that transplantation of bone marrow from the hereditary or spontaneous autoimmune lupus like mouse strain New Zealand Black into lethally irradiated mice of a healthy DBA strain induced anti-nuclear antibodies and autoimmune disease in the recipient [9]. Subsequently it was shown that infusion of bone marrow (BM) derived from healthy donors could prevent autoimmune diseases after immunoablation of the host predisposed to develop autoimmune disease [10]. Following these observations, animals with induced forms of autoimmune diseases were treated with allogeneic or syngeneic (bone marrow from a healthy donor from the same strain) and (pseudo) autologous bone marrow transplantation (bone marrow from affected animals of the same strain). These models differed from the spontaneously developed forms since immunization was required with specific antigens. In the case of experimental autoimmune encephalitis (EAE), an animal model for multiple sclerosis, basic myelin protein and in adjuvant arthritis (AA) bacterial antigens such as mycobacterium tuberculosis were used for induction of disease. AA in Buffalo rats causes a chronic progressive type of polyarthritis with similar histopathology of the synovium as in RA with proliferating synovitis and pannus formation. In AA it was shown that an immunoablative regimen followed by allogeneic but unexpectedly also autologous and syngeneic SCT could not only prevent the disease but also induce remission (reviewed by Van Bekkum [11]). Spontaneous relapses after syngeneic bone marrow transplantation were rare in AA but did occur frequently in EAE (30% after syngeneic vs. 5% after allogeneic transplantation). The relapses could not be explained by adoptively transferred T cells as syngeneic bone marrow is derived from a healthy donor from the same strain not immunized with basic myelin protein. Therefore, the cells responsible for the relapse could be residual T cells that survive the immunoablative regimen. AA and EAE are considered T cell diseases since adoptive transfer of T cells from diseased animals could mediate disease in healthy animals [12;13], but genetic factors also play an important role in the susceptibility and/or resistance to disease, as only genetic susceptible strains develop disease. Nonetheless, the findings described above underline the significance of bone marrow derived cells in these forms of autoimmune diseases, but do not point to intrinsic abnormality of stem cells. Therefore these experiments suggest that 1. myeloablative therapy followed by bone marrow transplantation may induce remissions, 2. allogeneic transplantation may be more effective than autologous transplantation in vivo and 3. T-cell depletion of the graft may be beneficial.
Human autoimmune diseases are considerably more complex and heterogeneous than animal autoimmune diseases. Based upon the lessons made in animal models, the key issue in *humans* is whether autoimmune diseases are due to intrinsic hematopoietic stem cell defects or defects at the level of more mature lymphoid populations (e.g. wrongly

programmed lymphoid cells reactive to self antigens). It is therefore difficult to extrapolate the results of the animal studies to human rheumatic diseases.

Allogeneic bone marrow transplantation and pre-existing autoimmune disease.

It was shown that transplantation of bone marrow from patients suffering from an autoimmune disease could transfer autoimmune symptoms to the donor in myasthenia gravis [14], autoimmune thyreoiditis [15] and insulin dependent diabetes mellitus [16]. However, adoptive transfer of bone marrow cells from a donor with RA did not induce disease [17], neither was disease induced by bone marrow from a donor with systemic lupus erythematosus (SLE) in a recipient [18]. It could be mooted that pathophysiological differences exist between systemic- vs. organ specific- autoimmune diseases. Of particular interest are patients with autoimmune diseases who were treated with allogeneic bone marrow transplantation because of a concomitant hematological malignancy or severe aplastic anaemia. Most cases involved patients who received allogeneic bone marrow transplantation because of aplastic anaemia due to gold and/or D-penicillamine therapy. Of the 8 reported cases, the first three patients, transplanted in the early days of bone marrow transplantation died due to transplant related complications, but attained a remission immediately following BMT. The other patients were free of symptoms (one patient experienced a temporary relapse of disease activity) with a follow-up ranging from 2 to 8 years [19-23], although one RA patient receiving an allogeneic bone marrow transplantation for aplastic anaemia with initial remission, recurrence and progression of RA occurred after 2 years, despite a full donor engraftment [24]. Together these data support the favorable effect of allogeneic BMT on RA in a majority of patients.

Autologous hematopoietic SCT for RA.

There are only two reports of patients with RA treated with autologous transplantation for a Non-Hodgkin lymphoma [25;26]. Both patients experienced relapse of RA 5 weeks and 20 months after autologous SCT. The occurence of relapse in these patients was attributed to the fact that the stemcell product was not depleted of potentially autoreactive lymphocytes and were therefore reinfused in the patient which subsequently could be responsible for a relapse in the two patients.

International Registry.
In 1995 the European Group for Blood and Marrow Transplantation (EBMT) and the European League against Rheumatism (EULAR) developed guidelines entry criteria and transplant treatment protocols for severe autoimmune diseases. The lack of alternative treatment options for severe, uncontrolled autoimmune diseases and poor prognosis in subgroups of patients with RA prompted development of this treatment strategy. Until recently, mortality and morbidity of blood or marrow SCT appeared too high to justify the risk of such a procedure in patients with RA alone. For example, myeloablative chemo(radio) therapy and *allogeneic* transplantation are associated with a transplant related mortality of 15-30%. In contrast, *autologous* SCT for patients with malignancies is associated with a transplant related mortality of less than 5% [27-29]. Although data from animal studies suggested that allogeneic SCT might be highly effective in treating human autoimmune disease, mortality and morbidity of allografting using 'classic' conditioning regimens can not be justified in a chronic disease such as RA in which prevention of morbidity instead of mortality is the major goal.

Study protocol.
As no experience existed with patients treated with autologous stem cells or bone marrow in the autoimmune setting a cautious approach was proposed. However several issues had to be addressed regarding the completion of an exact study protocol: mode of stem cell collection, necessity of T cell depletion of the hematopoietic stem cell transplant and the composition of high dose immunosuppressive regimen.

Stem cell collection.
Autologous stem cells can be isolated from peripheral blood. Although the number of progenitor cells in normal peripheral blood is low compared to bone marrow [30], successful mobilization of hematopoietic progenitor cells from bone marrow into peripheral blood can be achieved by the use of hematopoietic growth factors, such as granulocyte-colony stimulating factor (G-CSF), with or without priming with cyclophosphamide [31]. It was unknown whether mobilization of stem cells was possible in RA patients in which an intrinsic stem cell defect could prohibit successful mobilization. Burt et al showed that stem cells can be collected successfully in patients with autoimmune diseases including rheumatoid arthritis [32]. In this study it was shown that G-CSF used in combination with cyclophosphamide caused fewer flares than G-CSF alone and resulted in higher stem cell yield. The preliminary experience so far suggests that following 5-10 days of G-CSF treatment, sufficient numbers of stem cells can be harvested from blood in a single or two consecutive leukapheresis sessions. It has been demonstrated in several clinical studies on hematological malignancies, that peripheral blood stem cells (PBSCs), using G-CSF and cyclophosphamide induce a more rapid

reconstitution of hematopoiesis when compared to autologous bone marrow [33;34]. Thus, mobilization of PBSCs using cyclophosphamide and G-CSF seemed a logical choice to improve disease activity after stem cell mobilization and shorten the aplastic period.

T cell depletion of the graft.

Based on the pivotal role of T cells in the pathogenesis of RA, T-cell depletion of the graft would be required to prevent reinfusion of autoreactive T and B cells. Several methods have been reported to effectively deplete T cells from the marrow or blood cell graft, including the administration of anti-T cell monoclonal antibodies (either with or without complement), counterflow centrifugal elutriation, sheep erythrocyte/T cell rosette formation or an application consisting of selection of CD34 positive cells [35]. The selection based on the expression of the CD34 antigen on hematopoietic progenitor cells can be used to isolate hematopoietic (CD34 positive) stem cells, which results in the depletion of CD34 negative cells including the mature T cells.

High dose immunosuppressive regimen.

The aim of high dose immunosuppressive regimen is to eradicate the cells of the immune system that are responsible for rheumatoid arthritis. Lymphoablative or myeloablative regimens can be employed. The latter regimens are more toxic, but may be more efficacious, since the etiology of rheumatoid arthritis is not known and stem cells may play an essential role. Because of safety concerns, as mentioned above, most study centers used high dose cyclophosphamide as a means to achieve a lymphoablative or severe immunesupressive regimen.

Aims

Based on the above mentioned considerations we conducted an open study to assess feasibility, safety, and efficacy of high-dose chemotherapy (cyclophosphamide 200 mg/kg) and CD34+ selected autologous hematopoietic stem cell transplantation in patients with rheumatoid arthritis. Furthermore we were interested to evaluate how immunological parameters were associated with clinical outcome and what the effects of high dose chemotherapy (HDC) + autologous stemcell transplantation (ASCT) were on synovial tissue, to gain insight in the immunopathology of HDC + ASCT in RA. Not only disease activity, but also joint damage, health assessment and patient preference were investigated.

Outline of the thesis.

The **second chapter** examines how HDC and ASCT compares to traditional antirheumatic therapies in terms of potential risks and benefits. HDC + ASCT is a treatment modality with potential morbidity and mortality. Because of a lack of controlled trials it is unknown whether the potential therapeutic benefits of HDC + ASCT outweigh the risks. Before embarking on a study of HDC + ASCT in patients with refractory RA we performed a decision analysis to evaluate the effects of long-term improvement of disease activity versus adverse side effects such as hospitalization and treatment related mortality (TRM) on Quality of Life (QoL).

In the **third chapter** we conducted a survey among patients with RA and rheumatologists to evaluate whether the risks of an aggressive therapy are acceptable and whether risk taking by patients was associated with disease characteristics, socio-economic parameters and/or personality traits. **Chapter four** describes the clinical and immunological effects of high dose chemotherapy followed by autologous hematopoietic stem cell transplantation in a cohort of 14 patients with refractory rheumatoid arthritis. **Chapter five** describes the effect on progression of joint damage in patients with therapy-refractory, active, destructive rheumatoid arthritis treated with HDC and ASCT. In addition, the magnitude and duration of these effects was assessed. The study reported in **Chapter six** was undertaken to examine serially taken samples from synovial tissue and blood from RA patients treated with HDC + ASCT in an attempt to unravel pathogenetic mechanisms in RA. We used lineage specific markers to analyze cellular infiltrates in the synovium, as well as activation and inflammatory markers to assess disease activity at the tissue level. **Chapter seven** examines the role of IL-7 in immune reconstitution after HDC and ASCT. **Chapter eight** describes the effect of high dose chemotherapy on Quality of Life and **Chapter nine** is dedicated to the summary and conclusions of this study.

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