Chapter 5

High dose chemotherapy and syngeneic stem cell transplantation in a patient with refractory rheumatoid arthritis: poor response associated with persistence of host autoantibodies and synovial abnormalities

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**Background:** Immunoablative therapy combined with haematopoietic stem cell transplantation (SCT) is a possible treatment for patients with severe rheumatoid arthritis (RA).

**Case report:** A patient with rheumatoid factor positive, progressively erosive RA, refractive to treatment, was treated with high dose cyclophosphamide, followed by reinfusion of an unmanipulated peripheral blood graft derived from her identical twin sister. The clinical response was unsatisfactory, necessitating reinstitution of treatment with disease modifying antirheumatic drugs, which was associated with persistence of host serum autoantibodies and a cellular infiltrate in synovium, notably of plasma cells.

**Discussion:** The effectiveness of syngeneic SCT may be critically dependent on the degree of immunoablation achieved or on the composition of the graft.

**Case report**
A 44-year old female was diagnosed with rheumatoid factor positive, erosive rheumatoid arthritis at the age of 31. Despite treatment with (combinations of) disease modifying antirheumatic drugs (DMARDs) including methotrexate, sulfasalazine, gold, azathioprine, prednisone, oral cyclophosphamide, etanercept and infliximab, smoldering disease persisted, leading to progressive joint destruction. The patient underwent multiple orthopedic surgeries that included prosthetic surgery of the wrists, several small hand joints and her left ankle, arthrodesis of the cervical vertebrae I-II and of the right ankle and several operations of tendon sheets in both hands. When screened for stem cell transplantation she was treated with prednisone 7.5 mg per day, methotrexate 15 mg once weekly and infliximab 200 mg i.v. every eight weeks. Ethics committee approval and informed consent were obtained from the patient and her identical twin sister. Both sisters had identical blood groups (A+ and Rh+) and identical HLA haplotypes. Syngeneity was confirmed by DNA-testing showing that the sizes of 15 micro satellite DNA markers were concordant. The patient tested positive for IgG antibodies against CCP and parvovirus B19. The donor was healthy and negative for serum IgM rheumatoid factor and IgG antibodies against CCP and parvovirus B19. Disease activity was assessed with the 28 joint Disease Activity Score (1). Synovial tissue was obtained via arthroscopy before transplantation and 2, 3 and 6 months after transplantation. The patient
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was conditioned with i.v. cyclophosphamide, 50 mg/kg/day during four days (day -5 until –2, total dose 13.4 g) and i.v. methylprednisolone 2 mg/kg from day –5 to –1 followed by tapering doses of prednisone starting at 1 mg/kg per day. Peripheral stem cells from the donor were mobilized by filgrastim 10 µg/kg s.c. and collected by leukapheresis on day –1 and day 0. An unmanipulated syngeneic graft of 182*10^9 leukocytes containing 53% mononuclear cells and 769*10^6 CD34+ cells was administered.

Results

Before conditioning, infliximab, methotrexate and prednisone were discontinued which resulted in a disease flare (Figure 1). The conditioning regimen was well tolerated by the patient except for a transient blurred vision, probably related to the administration of high dose steroids. The nadir of neutrophils was on day 8 (0.016*10^9 /L). During hospitalization she had no joint complaints and she was discharged on day 14. After one month, disease activity flared accompanied by an acute phase response. Intra-articular injection with 80 mg of methylprednisolone into the left knee was ineffective and two months post-transplantation arthroscopic lavage of the knee including synovial biopsies was performed followed by a repeat arthroscopy three months after transplantation. Because of the high leukocyte counts (50.000/mm³) in the synovial fluid (SF) and the rise in ESR, both synovial fluid and tissue were cultured at both occasions but the results were negative. Prednisone was maintained at a dose of 7.5 mg/day and methotrexate was reinstituted at incremental doses 15 weeks after the transplantation because of persistent disease activity. The patient then was re-admitted because of disabling pain of her left knee. Clinical assessment and radiographic studies showed severe progressive osteo-arthritis leading to joint replacement surgery at six months post-transplant. Joint radiographs one year after transplantation showed progression of joint erosion in hands, feet, ankles and left elbow as well as loss of cartilage thickness in hand and feet joints and the right knee. In the two years following the transplantation the patient again underwent multiple joint and tendon operations. Rheumatoid factor and antibodies against CCP and parvovirus-B19 remained detectable during follow-up. After failure of adding leflunomide, parenteral gold and anakinra respectively to MTX, adalimumab was started alongside MTX with a moderate response. Figure 1 shows the course of clinical and laboratory parameters.
Flow cytometry and synovial tissue analysis

Peripheral blood cell counting by flow cytometry showed a rise in mononuclear cells two weeks after transplantation and a reciprocal drop in total leukocyte count. After one month mononuclear cell and total leukocyte counts had returned to baseline values (Figure 1). The proportion of naïve (CD45RA⁺) cells among CD4⁺ cells ranged from 22% to 46% during follow up (data not shown).

At baseline and 2, 3 and 6 months post-transplant synovial tissue was collected from the inflamed left knee. A marked synovial infiltration with neutrophils was accompanied by plasma cell infiltration on all occasions (Figure 2). Light chain staining excluded monoclonality of the synovial plasma cell population (not shown). Figure 3 shows peroxidase staining of CD3, CD5, CD38 and CD138 after three months.

Discussion

High dose chemotherapy followed by autologous stem cell transplantation has been evaluated as a treatment option for severe therapy refractory RA. Although promising short term results have been reported, disease relapsed in most patients (2). Of note, sensitivity to DMARD therapy seemed restored. Several causes for failure to achieve permanent remission with autologous SCT in RA patients have been postulated (3). Based on theoretical considerations syngeneic or allogeneic SCT could be more effective. A successful syngeneic stem cell transplantation in a rheumatoid factor negative patient was described before (4), with a reported complete remission of the disease of two years after the transplantation.

We attempted to induce remission in a patient with severe, rheumatoid factor positive, erosive RA by treating her with high dose chemotherapy followed by a syngeneic, unmanipulated stem cell transplantation from her healthy, identical twin sister. We postulated that an unmanipulated syngeneic graft might contain regulatory T- and B-cells while transfer of healthy stem cells would be more effective in rebuilding a non-autoaggressive immune system. Also, a previous small randomized trial showed that RA patients treated with high dose cyclophosphamide and unmanipulated autologous stem cell transplantation had fewer relapses than those who received a manipulated graft (6). The outcome in our patient was unfavorable, however. The disease course after high dose chemotherapy and syngeneic SCT was characterized by a rapid relapse of disease activity, a sustained acute phase response and persistence of RF and anti-CCP antibodies. Several factors may have contributed to this outcome. First, our patient was seropositive and after treatment rheumatoid factor, anti-CCP
and anti-parvovirus B19 remained positive suggesting host plasma cells were not eradicated by the high dose chemotherapy nor replaced by new donor-derived plasma cells from the graft which contained less than 1% CD19+ cells. This notion was supported by the persistence of plasma cell infiltration in the synovial tissue post transplant. Second, because of safety concerns in a heavily pretreated patient, we opted not to use antithymocyte globulin in the conditioning regimen. It is conceivable this may have resulted in insufficient reduction of autoreactive lymphocytes. Third, although the graft was enriched for mononuclear cells, granulocytes constituted a significant proportion of the graft. These cells may have been activated as a result of mobilization with G-CSF enabling them to home to the synovium and act as effector cells.

Because of the syngeneic setting of the transplantation, we were unable to detect chimerism after transplantation but the rapid rise in CD45RA+ CD4+ lymphocytes in the peripheral circulation within two weeks probably reflects a population of donor cells as this population has been shown not to recover after treatment of RA patients with high dose cyclophosphamide (5). In conclusion, our patient was treated with high dose chemotherapy and syngeneic stem cell transplantation but flared after one month. The marked neutrophilic synovitis, persistence of serum autoantibodies and plasma cell infiltration of the synovium may have contributed to the rapid flare of RA. Preliminary data suggest transplantation of an allogeneic graft may be more effective in eradicating host immune cells than an autologous or syngeneic graft by induction of a graft-versus-autoimmunity effect (6). Improved and less toxic conditioning schemes make SCTs, even allogeneic, applicable in an increasing group of patients suffering from rheumatic diseases refractory to conventional antirheumatic drugs and biologicals.
Reference List


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Figure 1
Clinical changes and peripheral blood leukocyte count before and after stem cell transplantation. Clinical and laboratory parameters, concomitant DMARD use and synovial biopsy timepoints before and after transplantation (A). Peripheral leukocyte count and mononuclear cell subset counts (flow cytometry) before and after transplantation (B).

Figure 2.
Hematoxilin and eosin staining (A-D) at baseline (A and B) and after two months (C and D) showing marked infiltration of plasmacells and neutrophils. Peroxidase staining (E-H) after two months for CD5 (E), CD38 (F), CD3 (G) and CD138 (H). Original magnification 100x (A and C, E-H) and 400x (B and D)
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Figure 1

A

B

- ESR (mm/hr)
- CRP (mg/L)
- RF (units/mL)
- CCP (units/mL)
- DAS28

* synovial biopsy
Figure 2

A

B

C

D

E

F

G

H