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Autologous stem cell transplantation in juvenile idiopathic arthritis : regaining immunological tolerance and arresting disease progression

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Chapter 1

General introduction



1. Autoimmunity and autoimmune disease

The main function of the immune system is to defend the host against infections. Two integrated lines of defense are activated upon infection with a microorganism. First, the innate (aspecific) immune system, which is composed of certain cell populations (including neutrophils, macrophages, natural killer (NK) cells), their (polymorphic) receptors for cellular ligands and soluble factors (*e.g.* chemokines, cytokines, complement factors). Second, the adaptive (specific) immune system, which is initiated in the lymph nodes and other peripheral lymphoid organs (*e.g.* spleen, Peyer's patches) after presentation of antigenic determinants or epitopes, recognized by hypervariable receptors on T- and B lymphocytes, and which is driven to proliferation and maturation by co-stimulatory molecules and lead to the secretion of *e.g.* immunoglobulins and cytokines (1;2). Diversity of the immune response is generated by random mutations of the genes for T- and B-cell receptors and antibodies. Adaptive immunity can clear most foreign antigens, either in soluble form or as part of microbes and establish a state of long-lived protection resulting from immunological memory. Unfortunately, the same adaptive immune system can generate memory responses against self-antigens, thus contributing to pathological processes like autoimmune diseases (AID). These misdirected immune responses are referred to as autoimmunity. Low degree of autoimmunity and autoreactive T cells per se are a normal phenomenon and may possibly have a physiological role (3). Autoimmune diseases are manifested when progression from "benign" autoimmunity to pathological autoimmunity occurs. Recognition, activation and clonal expansion of autoreactive lymphocytes are crucial steps in the pathogenesis of autoimmune diseases and depend on genetic (MHC as well as non-MHC genes) and epigenetic factors. The trigger which leads to (over)reactivity of autoreactive T cells is usually an infection or contact with macromolecules at the mucous membranes. Immunological tolerance is the basic property of the immune system that provides for self/non-self discrimination in order to protect the host from imbalance resulting in a reaction to self. Central tolerance is achieved by clonal deletion of T cells bearing T-cell receptors (TCRs) with high affinity for self antigens expressed in the thymus (Figure 1).

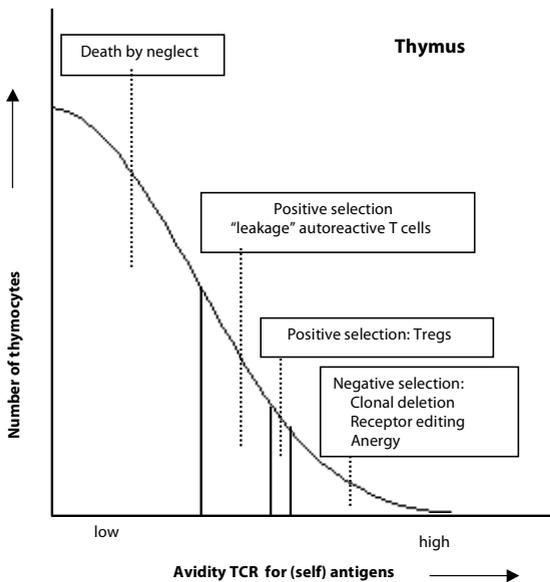


Figure 1. Central tolerance: positive and negative selection in the thymus

This central tolerance is not 100% guaranteed. Autoreactive lymphocytes which escaped selection in the central lymphoid organs, *i.e.* thymus and bone marrow, are present in the peripheral repertoire but are kept under control by lack of or inappropriate contact with the autoantigen and by a number of mechanisms that lead to peripheral tolerance. These mechanisms include anergy, phenotype skewing or activation-induced cell death of autoreactive T lymphocytes (1;2). Other mechanisms act through immature and/or tolerogenic dendritic cells as well as different types of regulatory cells, of which regulatory T cells (Tregs) play a dominant role (4-7). Several hypotheses attempt to explain the mechanisms by which tolerance can be broken, leading to self-reactivity. Infections can promote autoimmune responses by inducing tissue inflammation and therefore through bystander activation of autoreactive T cells or by promoting T-cell responses to microbial epitopes that cross-react with self peptides ("molecular mimicry"). Infections can lead to induction of costimulatory signals or may alter processing and expression of cryptic antigens, previously hidden from the thymic selection process (2). Although many issues have to be elucidated concerning autoimmunity and autotolerance, probably multiple overlapping pathways are operative in establishing, maintaining and breaking autotolerance.

2.1. Juvenile Idiopathic Arthritis

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in childhood, characterized by early onset arthritis (before the age of 16) that persists in one or more joints for at least 6 weeks. It is a heterogeneous group of chronic pediatric illnesses that share chronic joint inflammation

and have striking differences in severity and outcome (8-10). In the past, two main classification systems have been employed, *i.e.* the European League against Rheumatism (EULAR) criteria for Juvenile Chronic Arthritis (JCA) (11) and the American College of Rheumatology (ACR) criteria for Juvenile Rheumatoid Arthritis (JRA) (12;13). These criteria were not uniform, hampering meaningful comparison between the groups, and this has led to a taskforce of the International League of Associations for Rheumatology (ILAR) to develop new classification and diagnosis criteria for the idiopathic arthritides of childhood (14). The classification of different disease entities within the spectrum of JIA was introduced and revised several times by this committee (15;16) to achieve homogeneity within disease categories. Nowadays, JIA is divided in 7 subtypes based on clinical symptoms during the first six months of disease according to inclusion and exclusion criteria (Table I). The incidence of JIA has varied according to the literature from 2 to 20 per 100.000, with a prevalence of 16 to 150 per 100.000 (8). The etiology and pathogenesis of JIA are yet to be elucidated. However, there is indirect evidence that T-cell dysregulation plays a key role in the onset and probably also in the perpetuation of the disease (17;18). In this thesis children were studied with polyarticular JIA (pJIA) and systemic JIA (sJIA) in whom the course of the disease was progressive and not responding to available therapies.

Table I.

ILAR classification of Juvenile Idiopathic Arthritis

JIA subtype	Percentage of total
Systemic arthritis	10-15
Polyarthritis, rheumatoid factor negative	30
Polyarthritis, rheumatoid factor positive	3-5
Oligoarthritis a. persistent	40
b. extended	20
Enthesitis-related arthritis	1-7
Psoriatic arthritis	7
Unclassified arthritis	Undetermined

Adapted from Cassidy and Petty, 5th edition (8)

2.2. Current treatment in JIA

Until now there is no cure available for JIA. In general the aim of treatment, that combines antirheumatic drugs with physical and occupational therapy is straightforward, *i.e.* to try and achieve remission (19) with normal growth and development in a child and to prevent damage to the joints. The insights on medical treatment in JIA have changed during the last decade (10). Although the medical treatment depends to a large extent on the subtype of JIA, improved disease outcome has recently been achieved by early and aggressive immunosuppressive (combination) treatment, instead of by a gradual add-on approach of medication in case of ongoing disease activity. This approach is based on data obtained in RA patients (20;21). The prognosis for the majority of patients with JIA has also improved due to advances in treatment modalities, including

the use of sulphasalazine (22) methotrexate (23;24) and modulators of pro-inflammatory cytokines and their receptors (25-28). Despite better treatment results, a considerable number of children with polyarticular and systemic JIA remain refractory to therapy and have progressive disease with destruction of the joints as a consequence (8). Current treatments have to be improved, studies on the outcome in JIA show disappointing results with remissions ranging between 24 to 76% in 7 to 26 years of follow-up, depending on the onset type. Sustained drug free remissions are even harder to achieve. In the majority of patients with polyarticular disease the disease is active during almost two thirds of the follow-up (10;19;29-33). Disease progression will lead to destructive arthritis with severe joint deformities, growth retardation, and disability. The patient's health will further be compromised by the cumulative toxicity of anti-rheumatic medication (32;34). Whereas disease- and treatment- related mortality in JIA is now less than 1 % (8), the majority of deaths (65%) occur in the systemic onset group of JIA. This grim prospect for patients with severe JIA and the need for more effective treatments warrant experimental studies. Development of new curative strategies that block the continuation of autoimmunity or terminate the auto-immunological process remains a major challenge.

3.1. Background of autologous stem cell transplantation for children with severe JIA

It is thought that most AID are the consequence of a multifactorial process, mainly originating from the interplay of genetic, environmental and hormonal factors. Although the etiology and pathogenesis of JIA are not fully unraveled, there is convincing indirect evidence for a critical role of multiple factors in the immunopathological process, *i.e.* (unknown) triggers and autoantigens, possibly major histocompatibility complex (MHC) molecules, T cells (and their receptors, TCRs) and accessory cells (antigen presenting cells (APC's) such as macrophages and dendritic cells, and fibroblast-like synoviocytes). T cells seem to play a crucial role in the onset and probably in the continuation of juvenile arthritis (17;35). In the early stage of the disease, cellular infiltrate in the synovia mainly consists of T lymphocytes (36). Also the cytokines detected locally are T-cell derived (37-39). Studies with experimental arthritis and studies in children with a defective T-cell response to heat shock proteins (HSP) have shown a progressive polyarticular course of the rheumatic disease (40-42). The presentation of peptide antigens by susceptible human leucocyte antigen (HLA) molecules might be involved in the pathogenesis of JIA by activating autoreactive T cells. HLA-studies have not revealed strong and consistent associations in JIA as found in adult rheumatoid arthritis (RA). HLA class I and class II alleles are both associated with an increased risk to develop JIA. Early-onset oligoarticular JIA in girls is related to the class I antigen HLA-A2. Persistent and extended oligoarticular JIA are associated with class II antigens HLA-DRB1*08 and HLA-DRB1*11, DQA1*04, DQA1*05, and DQB1*04. Enthesitis-related JIA is associated with HLA-B27 (class I) and the class II antigens HLA-DRB1*01 and HLA-DQA1*0101. Systemic-onset JIA is related to HLA-DRB1*11 (43-45). The genetic predisposition also includes certain polymorphisms of genes that are related to cytokine production and chemokine receptors (46-48).

An immunodysregulation of T cells together with APC's, which are supposed to play a pivotal role in the pathogenesis of JIA, may be redressed by an intensive course of immunosuppression and stem cell rescue, as first proposed by Marmont for the treatment of systemic lupus erythematoses (SLE) (49;50). This concept evolved from coincidental observations in patients with an autoimmune disease, who received hematopoietic stem cell transplantation (SCT) for conventional indications. The autoimmune disease also responded to the SCT (51) and the ensuing hypothesis of resetting the T-cellular immunity by SCT was also tested extensively in animal studies, as described below.

3.2. Stem cell transplantation in experimental animal models of autoimmune disease

Experience with experimental animal models has been extensively reviewed (52;53). The animal models of autoimmune disease are of two different types; either "spontaneous" or "induced" models, *i.e.* either directly hereditary or with a genetic predisposition requiring environmental or external induction of autoimmunity (52-56). It has been shown by Ikehara et al. (57) and van Bekkum et al. (58) in both animal models that allogeneic SCT resulted in relief of symptoms of autoimmune diseases. These studies demonstrated that high-dose immunosuppressive therapy followed by the development of a new, donor-derived, immune system might be a therapeutic option in AID. Of key importance for the currently operative clinical protocols were the observations that improvement of disease symptoms could also be obtained after (pseudo)autologous SCT in the induced model of adjuvant arthritis (AA) (59). AA can be induced in susceptible Buffalo rats by intracutaneous injection of *Mycobacterium tuberculosis* in incomplete Freund's adjuvant. This causes a progressive type of polyarthritis showing inflammatory proliferative synovitis, destruction of cartilage and pannus formation, thus sharing characteristics with human Rheumatoid Arthritis (RA). Knaan et al. (59) unexpectedly showed that established arthritis could be abrogated by syngeneic and autologous SCT (ASCT) and with no recurrence of disease after rechallenge with infused T cells of arthritic animals. The curative effect of ASCT was demonstrated in rats with induced AA and in basic myelin protein induced experimental allergic encephalomyelitis (EAE) (60;61), as a model for human multiple sclerosis. The success of ASCT in rats depended on the completeness of eradication of antigen-specific activated and memory T cells in the graft and the host by T-cell depletion of the graft and the conditioning regimen of the host, respectively (Figure 2). The best results were obtained with supralethal doses of TBI or with a combination of lower doses of TBI and cyclophosphamide (see Figure 2). A significant difference was observed in the percentage of maintained responses between the groups treated with cyclophosphamide alone and those treated with a supralethal dose of TBI or the combined regimen (62). Interestingly, reimmunization, used as a positive control group, resulted in relapses in two of the 10 animals in the supralethal TBI group (Figure 2).

In contrast, if no T-cell depletion was performed, AID persisted in the recipient animals. These experiments showed that "auto"-reactive T cells present in the graft may result in continuation of the AID after ASCT, but that after stringent depletion of these cells curative effects similar to those obtained after allogeneic SCT can be achieved after ASCT.

No animal model is available for JIA. Many lessons have been learned from these AA animal studies, although extrapolation of these results to a heterogeneous disease as JIA is not obvious.

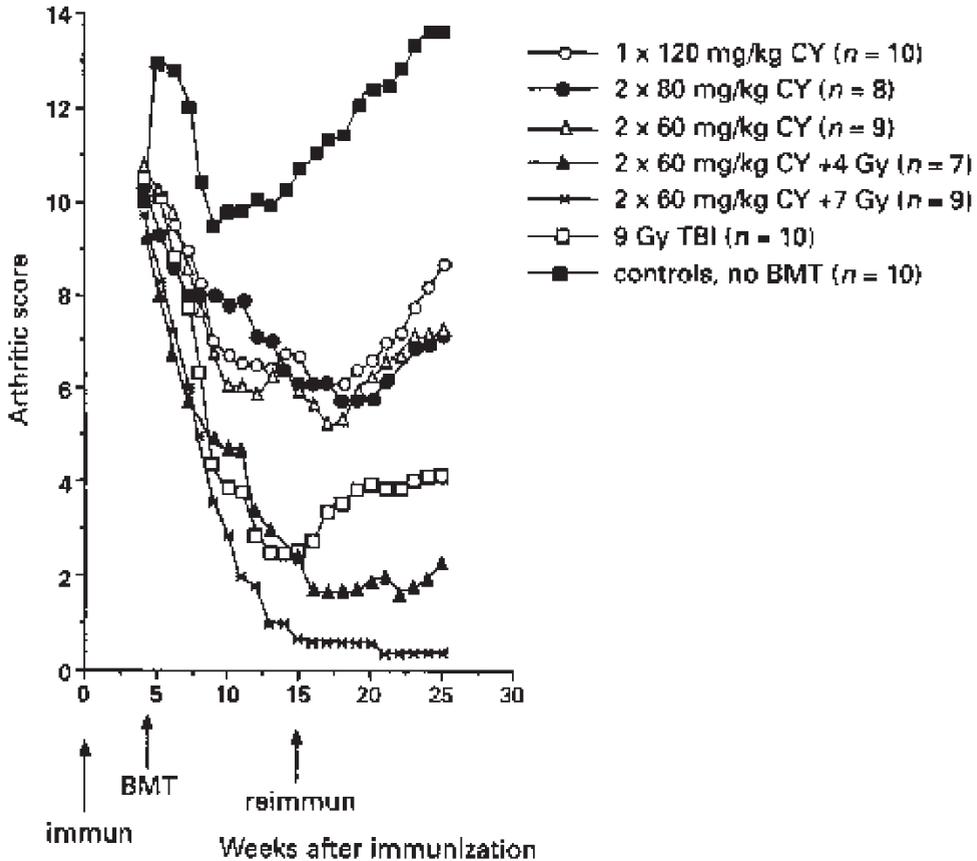


Figure 2. Conditioning with various doses of cyclophosphamide and combinations of cyclophosphamide with sublethal TBI in AA. Reprinted by permission from Macmillan Publishers Ltd: van Bekkum BMT 2000 (25), 357-364.

3.3. Allogeneic stem cell transplantation in patients with pre-existing autoimmune disease

Although hampered by a publication bias towards positive outcome, the information derived from allogeneic SCT in coincidental AID has encouraged utilization of ASCT for primary AID in the mid 1990s. A large number of single case studies, small case series and reviews on this topic have been published showing the outcome of coincidental AID in patients undergoing an allogeneic SCT for conventional indications as malignancies and severe aplastic anemia (SAA). Allogeneic SCT resulted in long-lasting remission of coincidental AID in many cases, but not in all (63-67). The greatest experience is available in iatrogenic gold induced SAA, considered to be, at least in part, an autoimmune disease itself. SAA has been treated successfully with allogeneic SCT for decades (68). In SAA patients, sensitized by multiple blood transfusions, an intensive conditioning, *i.e.* a combination of low dose TBI and cyclophosphamide, is needed to prevent rejection of the allograft (69;70).

4. Rationale for autologous stem cell transplantation for children with severe JIA

Both the studies in animal models and human patients reported above indicated that intensive myelo- and immunosuppressive therapy is capable of eradicating autoreactive T cells responsible for AID. In animal studies, hemopoietic reconstitution by allogeneic or T-cell depleted ASCT has been reported, without recurrence of the disease. In 1997, at the start of our study on ASCT in JIA, the very first case reports were published. Despite the limitations of a short follow-up at that time, successful results have been reported of ASCT for different AID (71-74). Although allogeneic SCT has been followed by sustained complete remissions, the substantial risk of morbidity and mortality associated with allogeneic SCT has so far prohibited its application on a large scale in the treatment of patients with AID. The choice for ASCT instead of allogeneic SCT was based on the following arguments: First, in general the frequency and severity of complication after allogeneic SCT are higher than after ASCT. The transplant related mortality (TRM) after allogeneic SCT is about 10-15%, compared to about 2-3 % after ASCT in conventional settings (75-79). Second, after allogeneic SCT chronic graft versus host (GVHD) may occur, which mimics AID in various aspects. Third, due to the use of higher TBI doses in allogeneic SCT in order to prevent graft rejection, long-term irradiation-induced effects on growth and gonads are more severe, which is especially relevant for children (80;81). Fourth, convincing data from animal studies show that ASCT may be as effective as allogeneic SCT in curing induced AID. After intensive therapy followed by rescue with autologous hematopoietic stem cells, purged of potentially autoreactive mature T lymphocytes, precursor T lymphocytes will differentiate and mature in the presence of the hypothetical autoantigen(s) and thus recognize the latter as "self". This may redress the balance between functionally different T-cellular populations leading to the "normal" non-autoreactive state.

5. Treatment protocols of the European Group for Blood and Bone marrow transplantation (EBMT) for ASCT in AID.

In 1995 the European Group for Blood and Marrow transplantation (EBMT) and the European League against Rheumatism (EULAR) developed guidelines for patient entry, source of transplant and manipulation of the graft and conditioning regimens (82;83). Many follow-up reports have been published since (84-87). At the start of our study only anecdotal patient data were available with respect to ASCT in AID. To date, more than 1000 patients worldwide, including more than 100 children, with several AID have been transplanted (86;88;89). Overall outcome depends on numerous variables, including type of autoimmune disease, disease severity, patient selection criteria, treatment procedure, and time point of ASCT (the earlier in the disease process, the better). Nowadays, phase I/II trials have justified moving forwards to phase III comparative randomised controlled studies, started within EBMT and international bone marrow transplant registry (IBMTR), for instance ASTIMS trial for multiple sclerosis (90), ASTIS trial for systemic sclerosis (91).

6.1. Our prospective study

For developing a study protocol, several issues had to be addressed regarding stem cell collection, T-cell depletion of the graft, composition of conditioning regimen, and patient selection. The starting point was that two conditions have to be fulfilled in order for ASCT to be possibly effective, *i.e.* the conditioning has to wipe out the (dysfunctional) memory T cells and the reinfused hemopoietic stem cell suspension has to be purged from mature “memory” T cells (92). In order to achieve these goals, the developed protocol included an immunosuppressive pre-treatment, for ablation of (autoreactive) T-cellular memory in the host, and elimination to a certain degree of mature T cells in the graft, in order to avoid reinfusion of a large number of autoreactive T cells (Table II) (62;93).

Table II. Conditioning scheme and TCD of the graft

	Days from ASCT									
	-9	-8	-7	-6	-5	-4	-3	-2	-1	0
Antithymocyte globulin (cumulative dose 20 mg/kg)	x	x	x	x						
Cyclophosphamide (cumulative dose 200 mg/kg)					x	x	x	x		
Total body irradiation (single fraction 4 Gy)									x	
TCD-ASCT (CD34 2-4 x 10 ⁶ /kg, CD3≥1 x 10 ⁵ /kg)										x

6.2. Conditioning regimen

The chosen lymphoablative conditioning regimen consisted of high dose cyclophosphamide, antithymocyte globulin and low to medium dose total body irradiation (TBI). This was based on experiences in the AA animal models and our previous experience with exactly the same conditioning regimen used in transfusion-sensitized children with SAA (69;92). The aim is to eradicate activated “memory” T cells. The characteristics of these cells and their sensitivity to various cytotoxic drugs are poorly defined. There are, however, some animal data suggesting differences between cyclophosphamide and irradiation in targeting memory T cells (62;94). High dose cyclophosphamide as a sole conditioning agent in AA resulted in higher percentages of relapses, compared to combination therapy with TBI (59;62). From the start of ASCT in AID, discussions took place about the optimal conditioning regimen for AID, including for JIA. A matter of debate was and still is whether or not to include low to medium dose TBI in the pre-treatment, because of concerns of the late effects, such as development of secondary malignancies, cataract and growth retardation (95-97). To what extent TBI itself, or the combination with immunosuppressive drugs used for ASCT in AID and/or disease related risks concerning DNA damage/repair after TBI can result in a higher incidence of secondary malignancies is not known (98). In our experience long term follow-up of SAA patients did neither show an adverse effect on physical growth, nor on normal

pubertal development, and so far no secondary malignancies were observed. Boys had a somewhat decreased testicular volume indicating germ cell damage (81;92). Alternatively, pre-treatment for SCT containing only cyclophosphamide, was found to be associated with an increased frequency of secondary malignancies in the course of time (99;100).

6.3. T-cell depletion of the graft

First the bone marrow graft was depleted of mature T cells by two different methods, either by negative selection of T cells by immunorosette-sedimentation conditions using specific monoclonals (anti-CD2 and anti-CD3) coupled to autologous red blood cells, or by positive CD34+ selection by CliniMACS (Miltenyi Biotec, Munich, Germany)(101). Since infections after ASCT and the possible risk of macrophage activation syndrome (MAS) (see section 6.4)(102) seemed to be correlated with the extent of T-cell depletion, it was decided in the course of the study to restrict T-cell depletion of the graft to the immunorosetting technique in order for the graft to contain between 1 and 5×10^5 T cells/kg. This amount of T cells in the graft was previously shown in animal experiments not to interfere with the immune-abrogative effect of the pre-treatment (see further) (103). Purification of the graft by CD34+ selection yielded about $\leq 1 \times 10^4$ T-cells/kg, and was therefore no longer applied.

6.4. Unexpected TRM: lethal macrophage activation syndrome (MAS)

The TRM rate after ASCT in JIA and other AID turned out to be higher than initially anticipated (1-3%) (79;104;105). In reality an overall 9% TRM was observed in AID (104). Two out of 22 children, both suffering from systemic JIA died in May 1999 of MAS (106-108), one in the Utrecht pediatric department; the other in the Leiden pediatric department, possibly induced by EBV reactivation and *S. epidermidis* bacteraemia, respectively (102). This resulted in an unacceptable TRM of 9% (109). In the same period another patient with systemic JIA died of MAS 17 days post transplantation, in the Hôpital Necker-Enfants Malades, Paris, possibly as a result of toxoplasmosis (110). MAS is a rare and potentially life-threatening complication of chronic rheumatic diseases of childhood, in particular of systemic-onset JIA. MAS has often been related to viral infections (*i.e.* EBV, CMV) or induced by drugs such as sulphasalazine, methotrexate and fludarabine (111-114). The onset is mostly acute and may mimic a flare of the disease or a severe sepsis. MAS is clinically characterized by fever, hepatosplenomegaly, lymphadenopathy, profound depression of all three major blood cell lines, deranged liver function, intravascular coagulation, and central nervous system dysfunction. It is thought to be caused by the activation and uncontrolled proliferation of T lymphocytes and macrophages, resulting in an unrestricted release of inflammatory cytokines (111;115-117). Laboratory data show pancytopenia, coagulopathy, low ESR, low concentrations of serum albumin, and high levels of ferritin, liver enzymes and triglycerides. Activated macrophages are found in various organs, particularly in bone marrow. Diagnosis is difficult, especially in an ASCT setting during pancytopenia, as was the case in one of our patients. Most hypotheses on the mechanism underlying MAS are based on observations in primary hemophagocytic lymphohistiocytosis (HLH), a genetic disease very similar to MAS, including decreased NK cell activity and decreased perforin expression in cytotoxic cells (118-120). Prompt diagnosis, and differentiation from sepsis, is essential

for outcome; prognosis is highly related to early specific treatment. Treatment consists mainly of intravenous methylprednisolone pulses and cyclosporine A (111).

After lethal MAS following ASCT, changes in strategy for further transplantations were discussed by the participating centers at the EULAR conference, Glasgow June 1999 and in Leiden July 1999. Circumstantial evidence pointed to a possible contributing role of the following variables:

- The subtype of JIA, *i.e.* systemic JIA (present in three cases)
- Signs and symptoms of systemic activity at the time of ASCT *i.e.* spiking fever, rash (present in 3 cases)
- Very stringent T-cell depletion of the graft by CD34 positive selection, in contrast to a less rigorous T-cell depletion by the immunorsetting technique (present in 3 cases).

The combination of these conditions might have led to the occurrence of MAS, following an infectious episode. The idea was that a too rigorous T-cell depletion, leaving 'no regulatory' T-cells in the graft for sJIA patients, susceptible for MAS (111), resulted in uncontrollable activation of macrophages following infection, leading to hemophagocytosis.

An international consensus was reached with respect to the transplant procedure and the surveillance after ASCT, resulting in an amended protocol for ASCT. It was decided to continue the study, applying more stringent inclusion criteria for sJIA patients, using additional parameters for surveillance after ASCT and indicating when and how to intervene for imminent MAS:

- Patients with active systemic disease (fever, rash), that cannot be controlled by steroids are excluded from the study.
- T-cell depletion is restricted to the immunorsetting technique, in order for the graft to contain at least between 1 and 5×10^5 T cells/kg (see section 6.3 T-cell depletion of the graft).
- Virological monitoring with RT-PCR methods from admission onwards until specific immunity is sufficiently recovered. Prophylaxis in each patient with (val)aciclovir may be considered, although this only gives a restricted antiviral coverage.
- Careful monitoring for emerging MAS and preemptive treatment for imminent MAS: in case of imminent MAS, *i.e.* spiking fever $\geq 39^\circ$ Celsius for 48 hours, besides taking blood cultures and performing additional investigations, start with methylprednisolone and cyclosporin A.

From 1999 onwards, 11 children (10 with sJIA) were treated with ASCT according to the amended protocol and no more MAS cases occurred.

Aims and outline of this thesis

This study was designed to evaluate the feasibility, efficacy, tolerability and safety of an immunoblative conditioning regimen followed by a T-cell depleted autologous stem cell reinfusion in severe JIA patients.

This thesis can be divided in two parts. In the **first part** the clinical results and outcome after ASCT for JIA are described.

In **chapter 2** we investigated in a prospective phase I/II study the feasibility, efficacy and safety and long-term outcome (with a follow-up of at least 4 years) of ASCT for JIA with application of an immunoablative pretreatment in 22 patients admitted in three different centers.

Chapter 3 is the report of a retrospective study showing the results of ASCT in 34 JIA patients from nine different European transplantation centers, obtained from the EBMT registry data, comparing 3 different conditioning regimens.

Chapter 4 describes the effects of conditioning on the composition of the cellular infiltrates and on the expression of proinflammatory cytokines in the synovial tissue in relation to the clinical status after ASCT.

The subject of **chapter 5** is the macrophage activation syndrome (MAS), which was an unexpected lethal complication early after ASCT for JIA and lead to an amendment of the study protocol. Here a case report of a boy with systemic JIA, treated with ASCT and suffering from lethal MAS as a transplant-related complication, is described.

The potential effect of ASCT in autoimmune diseases is thought to be the result of eradication of immunological memory by the immunoablative pre-treatment, and resetting of the adaptive immunity after T-cell depleted ASCT. This hypothesis can only be studied indirectly, using test antigens, because the exact nature of the eliciting antigens in autoimmune diseases is unknown. Therefore, in the **second part** of this thesis we studied the effect of the immunoablative conditioning on antigen specific humoral and cellular immune responses to a neoantigen rabies and a recall antigen tetanus, described in **chapter 7**.

As no data were available concerning the humoral (antigen specific immunoglobulin isotypes, IgG subclasses, avidity maturation) and cellular (antigen specific in vitro proliferation) immune response to a primary vaccination and a secondary/booster vaccination with the neoantigen rabies, we conducted a study, **chapter 6**, to obtain reference values of the anti-rabies specific immune responses in healthy adults.

A summary and general discussion is presented in **chapter 8**.

References

1. Janeway C.A., Travers P. Immunobiology:the immune system in health and disease. Garland Science Publishing, NY; 1997.
2. Delves PJ, Martin SJ, Burton DR, Roitt IM. Roitt's essential immunology. eleventh edition. Blackwell Publishing; 2006.
3. Samy ET, Setiady YY, Ohno K, Pramoonjago P, Sharp C, Tung KS. The role of physiological self-antigen in the acquisition and maintenance of regulatory T-cell function. *Immunol.Rev.* 2006;212:170-84.
4. de Kleer I, Vastert B, Klein M, Teklenburg G, Arkesteijn G, Yung GP et al. Autologous stem cell transplantation for autoimmunity induces immunologic self-tolerance by reprogramming autoreactive T cells and restoring the CD4+CD25+ immune regulatory network. *Blood* 2006;107(4):1696-702.
5. de Kleer IM, Wedderburn LR, Taams LS, Patel A, Varsani H, Klein M et al. CD4+CD25bright regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis. *J.Immunol.* 2004;172(10):6435-43.
6. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J.Immunol.* 1995;155(3):1151-64.
7. Zheng Y, Rudensky AY. Foxp3 in control of the regulatory T cell lineage. *Nat.Immunol.* 2007;8(5):457-62.
8. Cassidy JT, Petty, R. E., Laxer, R. M., and Lindsey CB. Textbook of Pediatric Rheumatology. 5th edition. Elsevier Saunders; 2006.
9. Wallace CA, Levinson JE. Juvenile rheumatoid arthritis: outcome and treatment for the 1990s. *Rheum.Dis. Clin.North Am.* 1991;17(4):891-905.
10. Wallace CA. Current management of juvenile idiopathic arthritis. *Best.Pract.Res.Clin.Rheumatol.* 2006;20(2):279-300.
11. Wood PHN. Special meeting on nomenclature and classification of arthritis in children. In:Munthe E (ed). The care of rheumatic children.EULAR, Basle 2007:47-50.
12. Brewer EJ, Jr., Bass J, Baum J, Cassidy JT, Fink C, Jacobs J et al. Current proposed revision of JRA Criteria. JRA Criteria Subcommittee of the Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Section of The Arthritis Foundation. *Arthritis Rheum.* 1977;20(2 Suppl):195-9.
13. Cassidy JT, Levinson JE, Brewer EJ, Jr. The development of classification criteria for children with juvenile rheumatoid arthritis. *Bull.Rheum.Dis.* 1989;38(6):1-7.
14. Fink CW. Proposal for the development of classification criteria for idiopathic arthritides of childhood. *J.Rheumatol.* 1995;22(8):1566-9.
15. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J.Rheumatol.* 2004;31(2):390-2.
16. Petty RE, Southwood TR, Baum J, Bhattay E, Glass DN, Manners P et al. Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. *J.Rheumatol.* 1998;25(10):1991-4.

17. Grom AA, Hirsch R. T-cell and T-cell receptor abnormalities in the immunopathogenesis of juvenile rheumatoid arthritis. *Curr.Opin.Rheumatol.* 2000;12(5):420-4.
18. Burt RK, Slavin S, Burns WH, Marmont AM. Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: getting closer to a cure? *Blood* 2002;99(3):768-84.
19. Wallace CA, Ruperto N, Giannini E. Preliminary criteria for clinical remission for select categories of juvenile idiopathic arthritis. *J.Rheumatol.* 2004;31(11):2290-4.
20. Allaart CF, Goekoop-Ruiterman YP, Vries-Bouwstra JK, Breedveld FC, Dijkmans BA, Group FS. Aiming at low disease activity in rheumatoid arthritis with initial combination therapy or initial monotherapy strategies: the BeSt study. *Clin.Exp.Rheumatol.* 2006;24(6 Suppl 43):S077-S082.
21. Landewe RB, Boers M, Verhoeven AC, Westhovens R, van de Laar MA, Markusse HM et al. COBRA combination therapy in patients with early rheumatoid arthritis: long-term structural benefits of a brief intervention. *Arthritis Rheum.* 2002;46(2):347-56.
22. van Rossum MA, Fiselier TJ, Franssen MJ, Zwinderman AH, ten Cate R, Suijlekom-Smit LW et al. Sulfasalazine in the treatment of juvenile chronic arthritis: a randomized, double-blind, placebo-controlled, multicenter study. Dutch Juvenile Chronic Arthritis Study Group. *Arthritis Rheum.* 1998;41(5):808-16.
23. Ruperto N, Murray KJ, Gerloni V, Wulfraat N, de Oliveira SK, Falcini F et al. A randomized trial of parenteral methotrexate comparing an intermediate dose with a higher dose in children with juvenile idiopathic arthritis who failed to respond to standard doses of methotrexate. *Arthritis Rheum.* 2004;50(7):2191-201.
24. Takken T, van der NJ, Helders PJ. Methotrexate for treating juvenile idiopathic arthritis. *Cochrane.Database.Syst.Rev.* 2001(3):CD003129.
25. Lovell DJ, Giannini EH, Reiff A, Jones OY, Schneider R, Olson JC et al. Long-term efficacy and safety of etanercept in children with polyarticular-course juvenile rheumatoid arthritis: interim results from an ongoing multicenter, open-label, extended-treatment trial. *Arthritis Rheum.* 2003;48(1):218-26.
26. Quartier P, Taupin P, Bourdeaut F, Lemelle I, Pillet P, Bost M et al. Efficacy of etanercept for the treatment of juvenile idiopathic arthritis according to the onset type. *Arthritis Rheum.* 2003;48(4):1093-101.
27. Pascual V, Allantaz F, Arce E, Punaro M, Banchereau J. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. *J.Exp.Med.* 2005;201(9):1479-86.
28. Yokota S, Miyamae T, Imagawa T, Iwata N, Katakura S, Mori M et al. Therapeutic efficacy of humanized recombinant anti-interleukin-6 receptor antibody in children with systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* 2005;52(3):818-25.
29. Wallace CA, Huang B, Bandeira M, Ravelli A, Giannini EH. Patterns of clinical remission in select categories of juvenile idiopathic arthritis. *Arthritis Rheum.* 2005;52(11):3554-62.
30. Zak M, Pedersen FK. Juvenile chronic arthritis into adulthood: a long-term follow-up study. *Rheumatology.(Oxford)* 2000;39(2):198-204.
31. Fantini F, Gerloni V, Gattinara M, Cimaz R, Arnoldi C, Lupi E. Remission in juvenile chronic arthritis: a cohort study of 683 consecutive cases with a mean 10 year followup. *J.Rheumatol.* 2003;30(3):579-84.
32. Gare BA, Fasth A. The natural history of juvenile chronic arthritis: a population based cohort study. II. Outcome. *J.Rheumatol.* 1995;22(2):308-19.

33. Flato B, Lien G, Smerdel A, Vinje O, Dale K, Johnston V et al. Prognostic factors in juvenile rheumatoid arthritis: a case-control study revealing early predictors and outcome after 14.9 years. *J.Rheumatol.* 2003;30(2):386-93.
34. Laxer RM. Long-term toxicity of immune suppression in juvenile rheumatic diseases. *Rheumatology.(Oxford)* 1999;38(8):743-6.
35. Grom AA, Giannini EH, Glass DN. Juvenile rheumatoid arthritis and the trimolecular complex (HLA, T cell receptor, and antigen). Differences from rheumatoid arthritis. *Arthritis Rheum.* 1994;37(5):601-7.
36. Silverman ED, Isacovics B, Petsche D, Laxer RM. Synovial fluid cells in juvenile arthritis: evidence of selective T cell migration to inflamed tissue. *Clin.Exp.Immunol.* 1993;91(1):90-5.
37. Eberhard BA, Laxer RM, Andersson U, Silverman ED. Local synthesis of both macrophage and T cell cytokines by synovial fluid cells from children with juvenile rheumatoid arthritis. *Clin.Exp.Immunol.* 1994;96(2):260-6.
38. Woo P. Cytokines and juvenile idiopathic arthritis. *Curr.Rheumatol.Rep.* 2002;4(6):452-7.
39. Mangge H, Gallistl S, Schauenstein K. Long-term follow-up of cytokines and soluble cytokine receptors in peripheral blood of patients with juvenile rheumatoid arthritis. *J.Interferon Cytokine Res.* 1999;19(9):1005-10.
40. de Kleer IM, Kamphuis SM, Rijkers GT, Scholtens L, Gordon G, de Jager W et al. The spontaneous remission of juvenile idiopathic arthritis is characterized by CD30+ T cells directed to human heat-shock protein 60 capable of producing the regulatory cytokine interleukin-10. *Arthritis Rheum.* 2003;48(7):2001-10.
41. Anderton SM, van der ZR, Prakken B, Noordzij A, van Eden W. Activation of T cells recognizing self 60-kD heat shock protein can protect against experimental arthritis. *J.Exp.Med.* 1995;181(3):943-52.
42. Prakken AB, van Eden W, Rijkers GT, Kuis W, Toebes EA, Graeff-Meeder ER et al. Autoreactivity to human heat-shock protein 60 predicts disease remission in oligoarticular juvenile rheumatoid arthritis. *Arthritis Rheum.* 1996;39(11):1826-32.
43. Forre O, Smerdel A. Genetic epidemiology of juvenile idiopathic arthritis. *Scand.J.Rheumatol.* 2002;31(3):123-8.
44. Prahalad S. Genetics of juvenile idiopathic arthritis: an update. *Curr.Opin.Rheumatol.* 2004;16(5):588-94.
45. Thomson W, Donn R. Juvenile idiopathic arthritis genetics - what's new? What's next? *Arthritis Res.* 2002;4(5):302-6.
46. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J.Clin.Invest* 1998;102(7):1369-76.
47. Prahalad S, Bohnsack JF, Jorde LB, Whiting A, Clifford B, Dunn D et al. Association of two functional polymorphisms in the CCR5 gene with juvenile rheumatoid arthritis. *Genes Immun.* 2006;7(6):468-75.
48. Rosen P, Thompson S, Glass D. Non-HLA gene polymorphisms in juvenile rheumatoid arthritis. *Clin.Exp. Rheumatol.* 2003;21(5):650-6.
49. Marmont AM. Immune ablation with stem-cell rescue: a possible cure for systemic lupus erythematosus? *Lupus* 1993;2(3):151-6.

50. Marmont AM. Immune ablation followed by allogeneic or autologous bone marrow transplantation: a new treatment for severe autoimmune diseases? *Stem Cells* 1994;12(1):125-35.
51. Marmont AM. Coincidental autoimmune disease in patients transplanted for conventional indications. *Best.Pract.Res.Clin.Haematol.* 2004;17(2):223-32.
52. van Bekkum DW. Stem cell transplantation for autoimmune disorders. Preclinical experiments. *Best.Pract. Res.Clin.Haematol.* 2004;17(2):201-22.
53. Ikehara S. Treatment of autoimmune diseases by hematopoietic stem cell transplantation. *Exp.Hematol.* 2001;29(6):661-9.
54. Ikehara S. Autoimmune diseases as stem cell disorders: normal stem cell transplant for their treatment (Review). *Int.J.Mol.Med.* 1998;1(1):5-16.
55. van Bekkum DW. Experimental basis of hematopoietic stem cell transplantation for treatment of autoimmune diseases. *J.Leukoc.Biol.* 2002;72(4):609-20.
56. van Bekkum DW. Stem cell transplantation in experimental models of autoimmune disease. *J.Clin. Immunol.* 2000;20(1):10-6.
57. Ikehara S, Good RA, Nakamura T, Sekita K, Inoue S, Oo MM et al. Rationale for bone marrow transplantation in the treatment of autoimmune diseases. *Proc.Natl.Acad.Sci.U.S.A* 1985;82(8):2483-7.
58. van Bekkum DW, Bohre EP, Houben PF, Knaan-Shanzer S. Regression of adjuvant-induced arthritis in rats following bone marrow transplantation. *Proc.Natl.Acad.Sci.U.S.A* 1989;86(24):10090-4.
59. Knaan-Shanzer S, Houben P, Kinwel-Bohre EP, van Bekkum DW. Remission induction of adjuvant arthritis in rats by total body irradiation and autologous bone marrow transplantation. *Bone Marrow Transplant.* 1991;8(5):333-8.
60. van Gelder M, Kinwel-Bohre EP, van Bekkum DW. Treatment of experimental allergic encephalomyelitis in rats with total body irradiation and syngeneic BMT. *Bone Marrow Transplant.* 1993;11(3):233-41.
61. van Gelder M, van Bekkum DW. Effective treatment of relapsing experimental autoimmune encephalomyelitis with pseudoautologous bone marrow transplantation. *Bone Marrow Transplant.* 1996;18(6):1029-34.
62. van Bekkum DW. Conditioning regimens for the treatment of experimental arthritis with autologous bone marrow transplantation. *Bone Marrow Transplant.* 2000;25(4):357-64.
63. Marmont AM. Stem cell transplantation for autoimmune disorders. Coincidental autoimmune disease in patients transplanted for conventional indications. *Best.Pract.Res.Clin.Haematol.* 2004;17(2):223-32.
64. Baldwin JL, Storb R, Thomas ED, Mannik M. Bone marrow transplantation in patients with gold-induced marrow aplasia. *Arthritis Rheum.* 1977;20(5):1043-8.
65. Snowden JA, Kearney P, Kearney A, Cooley HM, Grigg A, Jacobs P et al. Long-term outcome of autoimmune disease following allogeneic bone marrow transplantation. *Arthritis Rheum.* 1998;41(3):453-9.
66. McKendry RJ, Huebsch L, Leclair B. Progression of rheumatoid arthritis following bone marrow transplantation. A case report with a 13-year followup. *Arthritis Rheum.* 1996;39(7):1246-53.
67. Slavin S. Treatment of life-threatening autoimmune diseases with myeloablative doses of immunosuppressive agents: experimental background and rationale for ABMT. *Bone Marrow Transplant.* 1993;12(1):85-8.

68. Locasciulli A, van't Veer L, Bacigalupo A, Hovs J, van Lint MT, Gluckman E et al. Treatment with marrow transplantation or immunosuppression of childhood acquired severe aplastic anemia: a report from the EBMT SAA Working Party. *Bone Marrow Transplant.* 1990;6(3):211-7.
69. McCann SR, Bacigalupo A, Gluckman E, Hinterberger W, Hovs J, Ljungman P et al. Graft rejection and second bone marrow transplants for acquired aplastic anaemia: a report from the Aplastic Anaemia Working Party of the European Bone Marrow Transplant Group. *Bone Marrow Transplant.* 1994;13(3):233-7.
70. Srinivasan R, Takahashi Y, McCoy JP, Espinoza-Delgado I, Dorrance C, Igarashi T et al. Overcoming graft rejection in heavily transfused and allo-immunised patients with bone marrow failure syndromes using fludarabine-based haematopoietic cell transplantation. *Br.J.Haematol.* 2006;133(3):305-14.
71. Fassas A, Anagnostopoulos A, Kazis A, Kapinas K, Sakellari I, Kimiskidis V et al. Peripheral blood stem cell transplantation in the treatment of progressive multiple sclerosis: first results of a pilot study. *Bone Marrow Transplant.* 1997;20(8):631-8.
72. Marmont AM, van Lint MT, Gualandi F, Bacigalupo A. Autologous marrow stem cell transplantation for severe systemic lupus erythematosus of long duration. *Lupus* 1997;6(6):545-8.
73. Joske DJ, Ma DT, Langlands DR, Owen ET. Autologous bone-marrow transplantation for rheumatoid arthritis. *Lancet* 1997;350(9074):337-8.
74. Wulffraat N, van Royen A, Bierings M, Vossen J, Kuis W. Autologous haemopoietic stem-cell transplantation in four patients with refractory juvenile chronic arthritis. *Lancet* 1999;353(9152):550-3.
75. Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P et al. Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. *Bone Marrow Transplant.* 2005;36(9):757-69.
76. Bacigalupo A, Sormani MP, Lamparelli T, Gualandi F, Occhini D, Bregante S et al. Reducing transplant-related mortality after allogeneic hematopoietic stem cell transplantation. *Haematologica* 2004;89(10):1238-47.
77. Locasciulli A, Oneto R, Bacigalupo A, Socie G, Korthof E, Bekassy A et al. Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). *Haematologica* 2007;92(1):11-8.
78. Buadi FK, Micallef IN, Ansell SM, Porrata LF, Dispenzieri A, Elliot MA et al. Autologous hematopoietic stem cell transplantation for older patients with relapsed non-Hodgkin's lymphoma. *Bone Marrow Transplant.* 2006;37(11):1017-22.
79. Jantunen E, Itala M, Lehtinen T, Kuittinen O, Koivunen E, Leppa S et al. Early treatment-related mortality in adult autologous stem cell transplant recipients: a nation-wide survey of 1482 transplanted patients. *Eur. J.Haematol.* 2006;76(3):245-50.
80. Bakker B, Oostdijk W, Geskus RB, Stokvis-Brantsma WH, Vossen JM, Wit JM. Patterns of growth and body proportions after total-body irradiation and hematopoietic stem cell transplantation during childhood. *Pediatr.Res.* 2006;59(2):259-64.
81. Clement-De Boers A, Oostdijk W, Weel-Sipman MH, Van den BJ, Wit JM, Vossen JM. Final height and hormonal function after bone marrow transplantation in children. *J.Pediatr.* 1996;129(4):544-50.

82. Tyndall A, Gratwohl A. Blood and marrow stem cell transplants in autoimmune disease. A consensus report written on behalf of the European League Against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT). *Br.J.Rheumatol.* 1997;36(3):390-2.
83. Wulffraat NM, Kuis W, Petty R. Addendum: proposed guidelines for autologous stem cell transplantation in juvenile chronic arthritis. *Paediatric Rheumatology Workshop. Rheumatology.(Oxford)* 1999;38(8):777-8.
84. Tyndall A. Haematological stem cell transplantation in the treatment of severe autoimmune diseases: first experiences from an international project. *Rheumatology.(Oxford)* 1999;38(8):774-6.
85. Tyndall A, Musso M. Haematopoietic stem cell transplantation in the treatment of autoimmune diseases: current experience from an international data base. *Int.J.Artif.Organs* 2000;23(10):665-7.
86. Gratwohl A, Passweg J, Bocelli-Tyndall C, Fassas A, van Laar JM, Farge D et al. Autologous hematopoietic stem cell transplantation for autoimmune diseases. *Bone Marrow Transplant.* 2005;35(9):869-79.
87. Tyndall A, Daikeler T. Autologous hematopoietic stem cell transplantation for autoimmune diseases. *Acta Haematol.* 2005;114(4):239-47.
88. Tyndall A. Allogeneic bone marrow transplantation for autoimmune disease--the jury is still out. *J.Rheumatol.* 2006;33(4):644-6.
89. Hough RE, Snowden JA, Wulffraat NM. Haemopoietic stem cell transplantation in autoimmune diseases: a European perspective. *Br.J.Haematol.* 2005;128(4):432-59.
90. Fassas A. Autologous stem cell transplants in treatment of multiple sclerosis: where we stand and future prospects. *Int.J.Hematol.* 2002;76 Suppl 1:223-5.
91. van Laar JM, Farge D, Tyndall A. Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial: hope on the horizon for patients with severe systemic sclerosis. *Ann.Rheum.Dis.* 2005;64(10):1515.
92. Vossen JM, Brinkman DM, Bakker B, Hoogerbrugge PM, ten Cate R. Rationale for high-dose cyclophosphamide and medium-dose total body irradiation in the conditioning of children with progressive systemic and polyarticular juvenile chronic arthritis before autologous stem cell transplantation. *Rheumatology.(Oxford)* 1999;38(8):762-3.
93. Euler HH, Marmont AM, Bacigalupo A, Fastenrath S, Dreger P, Hoffknecht M et al. Early recurrence or persistence of autoimmune diseases after unmanipulated autologous stem cell transplantation. *Blood* 1996;88(9):3621-5.
94. Pestronk A, Drachman DB, Teoh R, Adams RN. Combined short-term immunotherapy for experimental autoimmune myasthenia gravis. *Ann.Neurol.* 1983;14(2):235-41.
95. Burt RK, Patel D, Thomas J, Yeager A, Traynor A, Heipe F et al. The rationale behind autologous autoimmune hematopoietic stem cell transplant conditioning regimens: concerns over the use of total-body irradiation in systemic sclerosis. *Bone Marrow Transplant.* 2004;34(9):745-51.
96. Urowitz MB, Rider WD. Myeloproliferative disorders in patients with rheumatoid arthritis treated with total body irradiation. *Am.J.Med.* 1985;78(1A):60-4.
97. Uhrin Z, Wang BW, Matsuda Y, Strober S, Genovese MC. Treatment of rheumatoid arthritis with total lymphoid irradiation: long-term survival. *Arthritis Rheum.* 2001;44(7):1525-8.
98. McCurdy D, Tai LQ, Frias S, Wang Z. Delayed repair of DNA damage by ionizing radiation in cells from patients with juvenile systemic lupus erythematosus and rheumatoid arthritis. *Radiat.Res.* 1997;147(1):48-54.

99. Ades L, Guardiola P, Socie G. Second malignancies after allogeneic hematopoietic stem cell transplantation: new insight and current problems. *Blood Rev.* 2002;16(2):135-46.
100. Radis CD, Kahl LE, Baker GL, Wasko MC, Cash JM, Gallatin A et al. Effects of cyclophosphamide on the development of malignancy and on long-term survival of patients with rheumatoid arthritis. A 20-year followup study. *Arthritis Rheum.* 1995;38(8):1120-7.
101. Slaper-Cortenbach IC, Wijngaarden-du Bois MJ, Vries-van Rossen A, Borst HP, van der LH, van Heugten HG et al. The depletion of T cells from haematopoietic stem cell transplants. *Rheumatology.(Oxford)* 1999;38(8):751-4.
102. ten Cate R, Brinkman DM, van Rossum MA, Lankester AC, Bredius RG, Egeler MR et al. Macrophage activation syndrome after autologous stem cell transplantation for systemic juvenile idiopathic arthritis. *Eur.J.Pediatr.* 2002;161(12):686.
103. Bekkum DW. Immune ablation and stem-cell therapy in autoimmune disease. Experimental basis for autologous stem-cell transplantation. *Arthritis Res.* 2000;2(4):281-4.
104. Tyndall A, Fassas A, Passweg J, Ruiz dE, Attal M, Brooks P et al. Autologous haematopoietic stem cell transplants for autoimmune disease--feasibility and transplant-related mortality. Autoimmune Disease and Lymphoma Working Parties of the European Group for Blood and Marrow Transplantation, the European League Against Rheumatism and the International Stem Cell Project for Autoimmune Disease. *Bone Marrow Transplant.* 1999;24(7):729-34.
105. Wulffraat NM, Brinkman D, Ferster A, Opperman J, ten Cate R, Wedderburn L et al. Long-term follow-up of autologous stem cell transplantation for refractory juvenile idiopathic arthritis. *Bone Marrow Transplant.* 2003;32 Suppl 1:S61-S64.
106. Ramanan AV, Schneider R. Macrophage activation syndrome -- what's in a name! *J.Rheumatol.* 2003;30(12):2513-6.
107. Cortis E, Insalaco A. Macrophage activation syndrome in juvenile idiopathic arthritis. *Acta Paediatr.Suppl* 2006;95(452):38-41.
108. Ravelli A, Magni-Manzoni S, Pistorio A, Besana C, Foti T, Ruperto N et al. Preliminary diagnostic guidelines for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. *J.Pediatr.* 2005;146(5):598-604.
109. de Kleer IM, Brinkman DM, Ferster A, Abinun M, Quartier P, van der NJ et al. Autologous stem cell transplantation for refractory juvenile idiopathic arthritis: analysis of clinical effects, mortality, and transplant related morbidity. *Ann.Rheum.Dis.* 2004;63(10):1318-26.
110. Quartier P, Prieur AM, Fischer A. Haemopoietic stem-cell transplantation for juvenile chronic arthritis. *Lancet* 1999;353(9167):1885-6.
111. Ravelli A. Macrophage activation syndrome. *Curr.Opin.Rheumatol.* 2002;14(5):548-52.
112. Ferreira RA, Vastert SJ, Abinun M, Foster HE, Modesto C, Olive T et al. Hemophagocytosis during fludarabine-based SCT for systemic juvenile idiopathic arthritis. *Bone Marrow Transplant.* 2006.
113. Ravelli A, Caria MC, Buratti S, Malattia C, Temporini F, Martini A. Methotrexate as a possible trigger of macrophage activation syndrome in systemic juvenile idiopathic arthritis. *J.Rheumatol.* 2001;28(4):865-7.
114. Hertzberger-ten Cate R, Cats A. Toxicity of sulfasalazine in systemic juvenile chronic arthritis. *Clin.Exp. Rheumatol.* 1991;9(1):85-8.

115. Mouy R, Stephan JL, Pillet P, Haddad E, Hubert P, Prieur AM. Efficacy of cyclosporine A in the treatment of macrophage activation syndrome in juvenile arthritis: report of five cases. *J.Pediatr.* 1996;129(5):750-4.
116. Stephan JL, Kone-Paut I, Galambrun C, Mouy R, Bader-Meunier B, Prieur AM. Reactive haemophagocytic syndrome in children with inflammatory disorders. A retrospective study of 24 patients. *Rheumatology.(Oxford)* 2001;40(11):1285-92.
117. Sreedharan A, Bowyer S, Wallace CA, Robertson MJ, Schmidt K, Woolfrey AE et al. Macrophage activation syndrome and other systemic inflammatory conditions after BMT. *Bone Marrow Transplant.* 2006;37(7):629-34.
118. Grom AA. Natural killer cell dysfunction: A common pathway in systemic-onset juvenile rheumatoid arthritis, macrophage activation syndrome, and hemophagocytic lymphohistiocytosis? *Arthritis Rheum.* 2004;50(3):689-98.
119. Grom AA. Macrophage activation syndrome and reactive hemophagocytic lymphohistiocytosis: the same entities? *Curr.Opin.Rheumatol.* 2003;15(5):587-90.
120. Wulffraat NM, Rijkers GT, Elst E, Brooimans R, Kuis W. Reduced perforin expression in systemic juvenile idiopathic arthritis is restored by autologous stem-cell transplantation. *Rheumatology.(Oxford)* 2003;42(2):375-9.

