Review

Immune ablation and stem-cell therapy in autoimmune disease
Experimental basis for autologous stem-cell transplantation

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Abstract

Treatment of rats suffering from florid chronic progressive systemic arthritis or from chronic remitting/relapsing encephalomyelitis with autologous bone marrow transplantation (BMT) is highly effective. This finding was unexpected as the genotype of the bone marrow largely determines the susceptibility of both spontaneous and induced autoimmune diseases in rodents. The success of autologous stem-cell transplantation depends on the completeness of eradication of the effectors of autoimmune disease, in other words activated and memory T lymphocytes. The reviewed experimental data, when translated to the clinic, indicate that the patients should be subjected to a conditioning regimen that induces maximal lymphoablation and that the autologous transplant has to be T-cell depleted.

Keywords: arthritis, autoimmune, autologous, conditioning, encephalomyelitis, rats, stem cells

Introduction

The pivotal role of the haematopoietic system was revealed by investigations in haematopoietic chimeras between rodent strains that are susceptible or resistant to various autoimmune diseases (for review [1]). Whether these chimeric animals develop autoimmune disease is generally dictated by the genotype of the bone marrow, which led to the hypothesis that the underlying defect resides in the haematopoietic stem cell [2]. These experiments were performed with many different models of autoimmune disease, but there is no consensus regarding which models are representative of the corresponding human disease.

The animal models of autoimmune disease are of two distinct types: the hereditary or spontaneous forms and the induced forms. In diseases of the first category, the symptoms develop with age in most or all of the members of a particular inbred strain of rodents. These models include systemic lupus erythematosus-like syndromes in mice, insulin-dependent diabetes mellitus in mice and rats, and a complex syndrome of arthritis/colitis/dermatitis in human leucocyte antigen-B27 transgenic rats. Genetic factors dominate in the pathogenesis of these diseases. The autoimmune diseases of the second category do not develop spontaneously, but require induction by immunization with specific tissues (eg brain in the case of experimental allergic encephalomyelitis [EAE], which serves as model of multiple sclerosis), with tissue components like collagen (ie collagen-induced arthritis), or with bacterial antigens like *Mycobacteria tuberculosis* (adjuvant arthritis [AA]). These diseases can only be induced in specific strains of rodents. Susceptibility and resistance to induction are largely genetically determined, as demonstrated by cross-breeding, but environmental influences are also involved.

AA = adjuvant arthritis; BMT = bone marrow transplantation; EAE = experimental allergic encephalomyelitis; TBI = total body irradiation.

The aetiology of human autoimmune diseases is multifactorial, the dominant contributors being genetic as well as environmental. Thus, it is likely that the inducible autoimmune diseases represent the more realistic models for preclinical investigations. The curative potential of autologous BMT was demonstrated in rats with induced arthritis and induced encephalomyelitis. Other animal models have not been explored so far.

Treatment of full-blown autoimmune diseases with BMT

The experiments in haematopoietic chimeras had a logical sequel in attempts to cure animals with overt autoimmune diseases by replacing their bone marrow with that from an allogeneic donor of a normal or a noninducible strain. The myeloablation and lymphoablation that is required to achieve complete allogeneic chimerism is also highly effective in reducing the burden of autoreactive lymphocytes. Accordingly, allogeneic BMT after high-dose total body irradiation (TBI) was found to cure hereditary as well as inducible forms of autoimmune disease. Following these publications (for review [3]), the hospital records of long-term survivors of allogeneic BMT, as treatment for an haematological malignancy or aplastic anaemia, were searched for patients with coexisting autoimmune diseases at the time of the transplantation. A total of 21 patients were identified, comprising a wide range of autoimmune diseases, which went into complete remission in all of these patients [4,5]. Obviously, aplastic anaemia is itself an autoimmune disease that has been treated succesfully with allogeneic BMT for several decades.

On the other hand, the prevailing concepts at that time did not foster the idea of using autologous BMT for treating autoimmune diseases, because of the dominant influence of the genotype of the bone marrow on susceptibility to experimental autoimmune disease. It was therefore a complete surprise that animals suffering from AA responded just as well to autologous and to syngeneic BMT as they did to grafting with allogeneic bone marrow [6••].

The substantial risk of morbidity and mortality associated with allogeneic BMT has so far prevented its application in the treatment of patients with severe autoimmune disease. In contrast, the lower risks of autologous transplants justified trying this approach in patients with severe autoimmune disease who were refractory to current treatments. Additional justification came from the reports [7] that revealed a very poor life expectancy of subgroups of patients with progressive refractory rheumatic disease. Furthermore, lymphoablation and myeloablation, followed by rescue with haematopoietic stem cells seemed a rational way to intensify the treatment with pulsed doses of cyclophosphamide that were under clinical investigation.

The treatment protocols of the current clinical studies on autologous BMT are based essentially on our findings in the two models of induced autoimmune diseases: AA and EAE (see below). These models have much in common with regard to the responses to treatment, but there are also considerable differences. In translating these results to the clinic, one can choose to adhere strictly to the disease specificities (eg apply the results of the AA model to treatment strategies for RA and SLE; and those obtained with EAE to treatment strategies for multiple sclerosis). This approach suffers from the restrictions imposed by the imperfections of the models. The other way to look at the results is to select from each model the parameters that appear to be most decisive for the outcome and to apply these to all clinical protocols until experience shows otherwise.

Outcome of treatment of fully developed autoimmune diseases with autologous BMT

Both AA and EAE were induced in rats of the inbred Buffalo strain. These animals develop a chronic progressive systemic arthritis and a chronic remitting/relapsing encephalomyelitis, respectively. In both diseases high-dose TBI (9–10 Gy) induces responses in all animals [6••].

In AA 70% are complete responders and 30% are partial responders. Both spontaneous and induced relapses are extremely rare in AA after high-dose TBI, but do occur after conditioning with cyclophosphamide [8•]

Rats with clinical EAE, as manifested by limb paralysis and/or paresis, respond to autologous BMT with a rapid complete regression of the neurological symptoms, but one or more spontaneous relapses occur in 30% [9••]. Reimmunization at the time that most animals have recovered from their last spontaneous relapse reinduces disease in 70% of the animals. When syngeneic rather than autologous bone marrow is used in EAE, the spontaneous relapse rate is the same [10], indicating that these relapses are initiated by autoimmune cells that survived conditioning. Accordingly, T-cell depletion of the graft does not diminish the spontaneous relapse rate. This does not imply that the composition of the autologous graft, especially of the lymphocyte subpopulations, is irrelevant. On the contrary, after the addition of autologous spleen cells to the bone marrow graft, the spontaneous relapse rate of EAE rose to over 90%. Furthermore, the T-cell content of bone marrow grafts of rats is 10 times less than that of human bone marrow grafts.

In both models, the best results have been obtained with the strongest lymphoablative/myeloablative regimens (ie supralethal doses of TBI or a combination of a lower TBI dose and cyclophosphamide). Irradiation of the affected tissues only (the central nervous system in the case of EAE, and the legs in the case of AA), or shielding of those tissues with irradiation of the rest of the body resulted at

best in limited and temporary remissions [6••,10]. Fractionated TBI was studied in the AA model and proved to be as effective as single-dose TBI, provided that the total dose was properly adjusted upwards [8•].

In both AA and EAE, conditioning with cyclophosphamide alone or busulfan alone at highest tolerated doses was clearly inferior to TBI. A combination of cyclophosphamide and busulfan was only slightly less effective than high-dose TBI. The combination of a lower dose of TBI (4 Gy) with cyclophosphamide (2 × 60 mg/kg) was as effective as the highest dose of TBI [8•]. The noteworthy features of cyclophosphamide as the sole conditioning agent in AA were not only the lower rate of complete remissions, but also the substantial incidence (36%) of spontaneous relapses. In contrast, among 155 AA animals treated with high-dose TBI or the combination regimens, only one relapse occurred.

Clinical relevance

The impressive results obtained with high-dose lymphoablative/myeloablative conditioning regimens are in accordance with the current concept that autoimmune diseases are maintained by activated T cells. These have to be inactivated or eliminated as much as possible in order to achieve complete and lasting remission. The animal experiments are equivocal in demonstrating that treatment with lower doses of the various cytotoxic agents results in incomplete responses and more relapses. It is therefore unlikely that so-called nonmyeloablative conditioning will be useful, unless the degree of T-cell ablation achieved can match that of current myeloablative regimens. It is obvious that the more vigorous ablation carries a higher risk of treatment-related morbidity and mortality. The optimal regimen needs to be defined in the clinic for each category of autoimmune disease.

The finding that cyclophosphamide as the sole conditioning agent is less effective than high-dose TBI or the combination regimens implies that less lymphoreduction is achieved with cyclophosphamide. Many clinical teams prefer the use of cyclophosphamide alone or combined with antilymphocyte globulin for conditioning. TBI is not favoured, mainly because it increases the risk of cancer. However, cyclophosphamide is an alkylating agent and is therefore carcinogenic, as is prolonged treatment with high-dose immunosuppressive agents (for review [11]).

How can the excellent results with autologous stem cells in animal models and the encouraging preliminary results with this modality in patients be explained? The current hypothesis is that the reconstitution of the immune system from a few haematopoietic stem cells represents a recapitulation of ontogenesis, with the acquisition of self-tolerance. This theory is based mainly on the experience with grafting of purified allogeneic stem cells, which may lead to full reconstitution of the immunological system in the absence of graft-versus-host disease.

The most intensive conditioning gives the best results because it eliminates the highest proportion of autoreactive T lymphocytes. It stands to reason that this effect will be undone if T cells in the autograft outnumber those surviving in the patient. A rough estimate of the surviving fractions is available only for TBI, and is between 0.1 and 0.01% for the most effective dose (ie 9–10 Gy) [11]. Because of these uncertainties it seemed prudent to begin clinical studies with maximally depleted autografts. The recommendation to graft no more that 10^5 T cells/kg [12] was based on the capabilities of current CD34 cell selection techniques. However, these also remove B cells, natural killer cells and macrophages, which is unnecessary and possibly harmful. Such rigorous depletion may result in a prolonged period of severe immunosuppression with risks of infections and lymphoproliferative malignancies. Data from the most sensitive model (EAE) suggest that 2×10^6 T cell/kg would still be safe, and this level can be achieved by specific depletion methodology.

Finally, there is the option of using allogeneic stem cells, which in the EAE rat minimizes relapses due to its graft-versus-autologous T-cell effect [13]. Considering the higher risks of transplantation-associated mortality, the clinical exploration of allogeneic BMT should be postponed until it becomes clear from ongoing studies with autologous stem-cell transplants which patients might benefit. For the treatment of connective tissue type autoimmune diseases, allogeneic stem cells cannot be recommended because graft-versus-host reactions are very difficult to distinguish from lesions due to the original autoimmune disease. It has been suggested that the induction of mixed allogeneic chimerism might be an attractive option, by virtue of a graft-versus-host autoimmunity effect in the absence of toxicity and graft-versus-host disease. Apart from the problem that such induction is as yet far from standardized, our results with mixed chimeras in EAE revealed a very high incidence of relapses [14].

For the time being it seems that the available animal models have been rather exhaustively employed for generating the essential preclinical data [15]. They are not suitable to sort out more subtle details for the optimization of treatment. The large variety of autoimmune diseases and the variation of disease manifestations within each disease cannot be imitated in much detail in the laboratory. If the accumulated clinical experience with autologous stem-cell transplants identifies specific fundamental questions, the animal models might again become useful.

Conclusion

The results of treatment with autologous BMT of the active inflammatory stages of EAE as well as of AA show that the

highest response rate and the lowest relapse incidence are achieved using the strongest lympho-myeloablative conditioning regimens. A similar approach for treating patients with refractory severe progressive autoimmune diseases is justified in view of the relatively low transplantation-related mortality of <5% as observed in leukaemic and aplastic patients who receive autologous BMT. This risk may well outweigh the mortality and the deterioration of quality-of-life associated with ineffective treatments. Ongoing clinical studies will establish the efficacy of autologous BMT, and accordingly the value of the animal models in preclinical research.

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