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Graft-Versus-Host Disease: Immunobiological Aspects*

P. J. Tutschka

Graft-versus-host disease continues to be a major problem in clinical allogeneic bone marrow transplantation. Despite great efforts to describe the disease clinically, histopathologically and immunologically we are far from understanding the immunobiology of graft-versus-host disease and transplantation tolerance, preventing us from developing rational clinical strategies to overcome this stumbling block.

In allogeneic bone marrow transplantation the lymphohematopoietic system of an adult, fully immunocompetent donor individual is transferred into a recipient that has been rendered immunologically incompetent, and the new graft is expected to survive, to function in an immunologically competent way yet to incorporate the transplantation antigens of the host into the already established repertoire of self, thus achieving a state of specific immunologic tolerance against the host [1–3]. Amazingly enough, such a seemingly impossible task can be achieved, and stable, immunologically competent yet specifically host-alloantigen tolerant chimeric states are reached in clinical and experimental bone marrow transplantation. However, in the majority of cases the complex and poorly understood process of "tolerization" is disturbed and the desired goal not achieved, resulting in the syndromes of acute and chronic graft-versus-host disease. Thus, despite almost 2 decades of clinical bone marrow transplantation, graft-versus-host disease in its acute and chronic form continues to be the major complication in marrow transplantation and a challenge for the clinician and the immunobiologist alike [4–7].

Graft-versus-host disease, to use a very simplistic operational definition, is considered to be the result of immunocompetent T-lymphocytes of donor origin that attack certain target organs of the host [8, 9]. Simonsen [10] and later Billingham [11] were the first to identify the requirements for a graft-versus-host reaction, a view that, at least superficially, is still valid for its practical implications:

1. The graft must contain immunologically competent cells.
2. The host must possess important transplantation isoantigens that are lacking in the graft donor, so that the host appears foreign to it and is therefore capable of stimulating it antigenically.
3. The host itself must be incapable of mounting an effective immunological reaction against the graft, i.e., the graft must have some security of tenure.

Based on that concept intensive research of more than 2 decades has attempted to establish the immunobiology of graft-versus-host reactions (GVHR), graft-versus-host disease (GVHD), and transplantation tolerance. In summary, the following statements were made:

1. The strength and kinetics of GVHR and GVHD are positively correlated with the degree of histoincompatibility.
2. The primary lesions of GVHD are the result of T-cell mediated cytotoxicity to target cells caused by the small, immunocompetent postthymic lymphocytes in the donor marrow inoculum.

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3. GVHR and GVHD are self-terminating immunologic events through deletion of host-responsive clones of donor cells.

Quite in accordance with these postulates were the results of intensive research into the immunobiology of marrow grafting that showed that the magnitude and kinetics of the graft-versus-host reactions (GVHR) were correlated with the degree of histoincompatibility between donor and recipient [1]. Transplantation tolerance, evolving after the resolution of graft-versus-host reactions and disease and characterized by immunologic unreactivity of donor cells toward host, but not third party alloantigens [3], developed rarely, in particular in mismatched donor-recipient combinations, and when it developed it was the result of a deletion or irreversible inactivation of specific clones of donor immunocompetent cells that were reactive against host alloantigens [7].

However, over the years several disconcerting observations [12, 13] were reported that cast some doubt on the validity of a concept that seemed so well founded by a plethora of experimental and clinical data. Recipients of marrow grafts that were mismatched not in major but minor histocompatibility antigens displayed graft-versus-host reactions and disease that were virtually indistinguishable in magnitude from those seen in major mismatches, a finding observed in humans, dogs, and rodents [14–17]. Even recipients of syngeneic grafts were described that showed graft-versus-host disease [18–21]. Furthermore, animals that were inbred and presumably of identical histocompatibility makeup showed marked differences in the incidence and severity of GVHD when grafted with marrow from the same histoincompatible inbred donor strain, some animals displayed no signs of GVHD clinically and histologically while others showed fatal GVHD [22]. Moreover, germ-free murine recipients of histoincompatible marrow showed no GVHD but developed the disease after intentional contamination with certain microorganisms [23, 24], suggesting that GVHD was not solely dependent on the degree of histoincompatibility between donor and host but rather required a trigger, most probably a mircoorganism. Viruses, in particular herpes type virus, a common complicating factor in clinical GVHD [19–29], have been incriminated in this trigger process, a postulate supported by the circumstantial evidence that GVHD could developed after a long lag period (up to 70 days in humans) and long after a seemingly complete and functioning lymphohemapoietic graft had been established [4, 8, 12]. These findings, seemingly inconsistent with the classical concept, led us to hypothesize that the complex immunological situation post-marrow grafting was not only dependent on the immunogenetically determined killer cells but on a counterforce of suppressor cells [3, 30, 31], that the magnitude and kinetics of the cytotoxic response were not determined by the antigeneic difference but by the magnitude of a suppressor cell response developing together with the cytotoxic cells. Transplantation tolerance, then, would not be seen as the result of a clonal deletion of alloantigen responsive cells but rather as a delicate balance between cytotoxic and suppressor cells whereby ultimately the suppressor cell arm gains preponderance, resulting in the stable, tolerant chimera [30–32].

Over the past several years we were indeed able to show that the specific immunologic tolerance seen in stable chimeras was maintained by alloantigen-specific suppressor cells [33]. This suppressor cell system has been rather extensively characterized in our rat bone marrow transplant model. Histoincompatible (mismatched at the Rt 1 locus) bone marrow allografts were established in lethally irradiated rats. At various times after transplantation lymphoid cells were harvested, subjected to mixed lymphocyte cultures, and assayed for immunological tolerance and for suppressor cells in vitro and in vivo. Alloantigen nonspecific suppressor cells appeared in the chimera 40 days after grafting, coinciding with the resolution of graft-versus-host disease. When specific tolerance was finally achieved in vivo and in vitro, a process that required between 100 and 250 days, the nonspecific suppressor cells were replaced by nylon wool adherent T-lymphocytes that specifically suppressed host alloantigen responses and could adoptively transfer the suppression of GVHD, suggesting that indeed the balance called operational tolerance

was actively maintained by specific suppressor cells. Specific tolerance in the chimeras was maintained during the 2 years of follow-up; however, the numbers of suppressor cells declined until they could no longer be demonstrated in vitro. A complete clonal deletion of host-reactive cells, though, of either alloresponsive clones or suppressive clones, did not occur. Restimulation of suppressor cells was possible with host antigen either by adoptive transfer or by inoculation of chimeric animals, suggesting that a clonal reduction had taken place in the long-term chimera which was followed by an induced expansion of suppressor cells clones [34].

The above described experiments suggested that alloreactive clones of cytotoxic cells were present in chimeras, but operationally not expressed, a postulate supported by the results of fractionation studies. Spleen cells from long-term complete and stable chimeras that were specifically tolerant to host alloantigens in vivo and in vitro were passed through nylon wool columns. The nylon wool nonadherent cells were then stimulated with host type stimulator lymphocytes and regained their ability to proliferate and develop specific cytotoxic effector cells [34].

In summary, these data seemed to indicate that induction of transplantation tolerance required a complex process of killer and suppressor cell interactions ultimately resulting in the incorporation of previously "foreign" antigens into the repertoire of "self".

It was conceivable to assume that not only transplantation tolerance but also tolerance against self was the result of a similar mechanism. This meant that in an adult organism autoreactive potential killer cells were present which were prevented from proliferating by the action of autoreactive thymus-dependent suppressor cells, which like in the long-term allogeneic chimera were present in a clonally reduced state.

If that were the case there should be circumstances under which a true autoaggression against transplantation antigens would occur, where a syngeneic or autologous reaction could be demonstrated that was neither clinically nor histologically distinguishable from a graft-versus-host reaction.

Such heretic thoughts have been expressed before. Cohen et al. reported about autosensitization in vitro [35], Parkman et al. [36] identified a subpopulation of lymphocytes in human peripheral blood cytotoxic to autologous fibroblasts and later explained the lack of autoreactivity in murine spleen cells by the concomitant presence of suppressor and cytotoxic lymphocytes, a view supported by the studies of L'age- Stehr and Diamantstein [37]. Gozes et al. finally induced a "syngeneic GVHR" in popliteal lymph nodes by spleen cells of old C57 B1/6 mice [38].

Clinically severe graft-versus-host disease has been described repeatedly in recipients of syngeneic bone marrow grafts [18–21]. Although apparently a rare occurrence in clinical transplantation, the reports suggested that "syngeneic GVHD" was neither an oddity nor the result of a transfusion accident (e.g., the infusion of an unirradiated blood product). Moreover, the factual presence of a "syngeneic GVHD" suggested that such a situation could be explored to understand better the nature of self-tolerance and disease states where self-tolerance was disturbed.

The bone marrow inoculum to be transplanted into a syngeneic, lethally irradiated recipient should contain autoreactive cytotoxic as well as autoreactive suppressor cells. Removal of suppressor cells by appropriate separation techniques prior to marrow infusion should result in syngeneic GVHD. Indeed, when suppressor cells were removed by either nylon wool fractionation or chemoseparation with 4-hydroxyperoxyclyclophosphamide we were able to create even syngeneic GVHD in lethally irradiated rat recipients [39, 40].

Acute graft-versus-host disease, then, could be seen as a disturbance of the tolerization process where either the cytotoxic effector arm is enhanced or the suppressor arm is either diminished or absent, leading to the observed injuries.

Chronic GVHD, in contrast, appears to present as an even more complex immunobiological situation. Rats with chronic GVHD, when evaluated immunologically, show an immunodeficiency, primarily of the T-cell arm, resulting in prolonged survival of third party skin grafts, depression of the antibody response to sheep red

blood cells, and impairment of the proliferative response of lymphocytes to alloantigens of host and third party strains. When assaying for suppressor cells we found that spleen and peripheral blood contained abundant numbers of alloantigen nonspecific suppressor T-lymphocytes that suppressed proliferative responses of original donor type to original host and third party alloantigens [41–45]. Adding such spleen cells to normal donor marrow inocula prior to transfer into secondary hosts not only prevented the development of acute GVHD but led to the rapid establishment of chronic GVHD clinically and histologically within 4 weeks after cell transfer. Speculating that nonspecific suppressor cells were causally involved in the pathogenesis of chronic GVHD, we harvested nonspecific suppressor T-cells from the spleens of healthy bone marrow chimeras early (48 days) after transplant and added them to normal donor marrow inocula prior to transfer into secondary hosts. Again, acute GVHD was prevented, but chronic GVHD developed within 4 weeks. Finally, we implanted thymuses from rats with chronic GVHD into normal rats that were lethally irradiated and reconstituted with donor type marrow immediately before thymus implantation. Those animals not only developed acute GVHD but also chronic GVHD within the first 4 weeks of marrow grafting [22, 34, 44, 45].

Thus chronic GVHD, at least in the rat model, again seemed to represent an imbalance of immunologically active cells, but unlike acute GVHD, not a relative or absolute decrease in the number of alloantigen-specific suppressor cells, but rather an increase in the number of alloantigen nonspecific suppressor cells under the influence of a malfunctioning thymus.

These concepts developed in animal models have been examined in the clinical bone marrow transplant situation, and some new strategies for the prevention and treatment of GVHD have been suggested. Patients who received an allogeneic bone marrow transplant, engrafted successfully, and have no evidence of GVHD, do indeed show suppressor T cells specific for host alloantigens [46]. Patients with acute GVHD lack those suppressor cells, whereas patients with chronic GVHD show large numbers of alloantigen nonspecific suppressor cells [46, 47].

Attempts to engineer the immunobiological situation after marrow transplantation and to facilitate the development of specific suppressor cells are encouraging. An agent of particular interest for this task is cyclosporin A, a fungal polypeptide that in vitro prevents the maturation of cytotoxic effector cells yet permits the development of suppressor effector cells [48, 49]. After very successful animal studies [49, 50], this agent is now used clinically to prevent acute GVHD. A pilot study performed in our institution has shown that this promising agent indeed prevented clinically severe GVHD in a majority of patients [51]. However, the study has also identified side effects of the new agent leading to clinically relevant complications, in particular, renal failure.

It is hoped that a better understanding of the pharmacology of this agent will lead to an improved utilization of the agent clinically, as well as further our understanding of transplantation tolerance in the recipient, thus widening the clinical applicability of bone marrow transplantation.

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