Leaders

Current thoughts on the pathogenesis of graft versus host disease

A L Appleton, L Sviland

Introduction

Bone marrow transplantation is increasingly being used to treat a variety of malignant and non-malignant diseases.^{1,2} As the toxicity of the conditioning regimen diminishes and the treatment of infections improves, graft versus host disease (GvHD) emerges as the most serious complication of allogeneic grafting. Acute GvHD typically occurs in 30–60% of recipients of allogeneic marrow grafts from HLA-matched sibling donors,³ and despite the introduction of immunosuppressive agents such as cyclosporin A and FK 506, it still has a mortality of up to 50%.⁴ Acute GvHD is difficult to eradicate and progression to chronic GvHD is associated with increased morbidity and mortality in long term survivors.

Manifestations of GvHD

Most cases of GvHD are seen after allogeneic bone marrow grafting, as a result of the transfer of a large number of allogeneic lymphoid cells into an immunocompromised recipient.

GvHD is also seen, however, in immunosuppressed patients who have been transfused with non-irradiated blood products,^{5,6} and in neonates with severe immune deficiency or very low birthweight due to the transplacental transfer of maternal T cells.^{7,8} It has also occasionally been described in recipients of certain types of solid organ grafts, notably small bowel and liver, and in this setting is thought to be the result of cells in the lymphoid areas of the transplanted organ mediating an allogeneic response against the recipient.^{9,10}

Typically, acute GvHD presents as a skin rash (fig 1), diarrhoea and abnormal liver function tests, with or without jaundice. Immunosuppression, which may be profound, is also a manifestation of GvHD.

Histological examination of the skin in acute GvHD shows focal or diffuse vacuolar degeneration of basal epidermal cells, with scattered eosinophilic bodies (degenerate keratinocytes) and an inflammatory infiltrate in the superficial dermis (fig 2).¹¹ The histological features of acute rectal GvHD are single cell necrosis of individual glandular crypt cells and a stromal inflammatory infiltrate (fig 3)¹²; similar features are seen in the duodenum and jejunum in upper gastrointestinal GvHD.¹³

Histological examination of the liver shows bile duct atypia, with necrosis of individual epithelial cells producing cytoplasmic vacuolation¹⁴; there may also be a sparse peribiliary inflammatory infiltrate, focal necrosis of hepatic parenchyma, and cholestasis.¹⁵

Figure 1 Typical diffuse erythematous maculopapular skin rash of GvHD, which predominantly affects proximal areas such as the upper arms, trunk, back (as shown) and face.

Figure 2 Histological features of acute cutaneous GvHD, showing pronounced diffuse vacuolar degeneration of basal epidermal cells and a prominent eosinophilic body (arrowed). There is a scanty inflammatory infiltrate in the superficial dermis.

Division of Pathology,
School of Pathological
Sciences, University
of Newcastle Upon
Tyne, Royal Victoria
Infirmary
A L Appleton
(née McGrehy)
L Sviland

Correspondence to:
Dr A L Appleton,
Department of Pathology,
Royal Victoria Infirmary,
Newcastle upon Tyne,
NE1 4LP.

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Figure 3 Histological features of acute rectal GvHD, with several glands showing single cell necrosis of individual glandular crypt cells (arrowed), and a scanty mixed cell inflammatory infiltrate within the stroma.

Aetiology of epithelial cell damage

The lesion common to GvHD in all affected organs is apoptosis of individual epithelial cells. This single cell necrosis is often, although not always, associated with a lymphocytic infiltrate. The epithelial damage associated with GvHD is in some way caused by donor effector cells reacting against recipient alloantigens,¹⁶ although the exact nature of the effector cell population in man remains a subject of controversy.

For reasons that are not fully understood, the principal target organs of GvH effector cells are the skin, gastrointestinal tract, hepatic biliary tree, bone marrow and lymphoid tissue. A possible reason for this apparent predilection for organs with a high cell turnover is that the continual expression of differentiation antigens by the cells of such organs triggers increased immune surveillance,¹⁷ although an alternative explanation is that the cells of these organs may harbour latent viruses which may act as a target for donor immune surveillance.¹⁸

Pathogenesis of GvHD

In man this is the subject of considerable controversy. Several theories based on experimental models and on clinical observations have been suggested. Few satisfactory animal models are available; unfortunately, GvHD behaves differently in rodents than it does in man. Primates probably offer the best animal model, but studies of GvHD in primates have proved difficult and expensive.

Although donor T lymphocytes are generally agreed to be the primary aggressors in establishing the graft versus host reaction,¹⁹⁻²¹ the pathogenesis of GvHD itself is far from clear. As long ago as 1971, Elkins made the distinction between the experimental graft versus host reaction (GvHR), which is the response of immunocompetent donor cells to the histocompatibility antigens of the immunocompromised recipient and which may be modified by a variety of immune and non-immune functions, and GvHD, which is the clinical manifestation of the GvHR.²² This area is still controversial and remains poorly understood.

Cytokines or direct cell damage?

The debate continues as to whether the epithelial damage mediated by GvH effector cells is directly inflicted by the cells themselves or, alternatively, mediated by cytokines released from these cells. Cytokines are regulatory molecules produced in response to an antigenic stimulus which form a complex network of communication between immunocompetent cells and which have a central role in T cell activation.

The role of cytokines in GvHD was first postulated in 1971. Streilein's "innocent bystander" theory proposed that the primary attack by donor effector cells is on host lymphocytes; this triggers the release of cytokines and results in "second-hand" damage to the skin and gastrointestinal tract—the "innocent bystanders".²³ This theory was later supported by the experimental animal work of others such as Elson and Mowat.²⁴⁻²⁵ The fact that the skin, gut, and liver have been shown to be particularly vulnerable to the effects of cytokines²³⁻²⁶ may further explain the targeting of these organs in GvHD.

Several groups have since studied the role of individual cytokines in the pathogenesis of GvHD. The T cell derived cytokine gamma interferon (γ IFN), which induces class II HLA antigen expression on epithelial cells,²⁷ has been implicated in GvHD. Aberrant expression of HLA class II antigens by keratinocytes and enterocytes is a feature of GvHD.²⁸⁻²⁹ Anti- γ IFN monoclonal antibody preparations have been shown to prevent the characteristic enteropathy of GvHD in mice.²⁶⁻³⁰

Tumour necrosis factor alpha (TNF α), which is a macrophage derived cytokine with multistimulatory effects on T cells and which is an important mediator of the inflammatory process, has also been implicated in GvHD.³¹⁻³³

In their study of a skin explant model of GvHD³⁴ Dickinson *et al* showed that TNF α causes histological changes in vitro similar to those seen in GvHD.³⁵ They also showed evidence of synergy between TNF α and γ IFN. These have already been shown to act together to produce local responses to antigenic stimuli.³⁶

The role of other cytokines, such as interleukin-2 (IL-2), in GvHD is less clearly defined. Several workers have found a correlation in bone marrow transplant recipients between GvHD and raised concentrations of the soluble form of the IL-2 receptor complex (s-IL-2R), which is shed from activated T cells into the serum.³⁷⁻³⁸ There is some evidence that monoclonal antibodies directed against IL-2R are effective at preventing murine GvHD,³⁹ although there is less convincing evidence for their clinical efficacy.⁴⁰⁻⁴¹

There is also evidence, however, that the cellular damage characteristic of GvHD may be caused by direct cell damage rather than indirectly through soluble mediators. Electron microscopic studies have shown evidence of intimate contact between effector cells and target epithelial cells in GvHR.⁴²⁻⁴⁴ These studies show that cytotoxic T cells may inflict tissue damage by direct cell to cell con-

tact. Small numbers of T cells may be able to produce clinically important damage in GvHD, because cytotoxic T cells may move from one target cell to another, destroying multiple epithelial cells.

There is also evidence that some direct cell killing may occur as a result of the activity of natural killer cells⁴⁵ which are non-HLA restricted. The histology of GvHD certainly supports this theory; acute GvHD affects immature cells, in both the basal layer of the epidermis in the skin and in the intestinal crypts. Studies of the anti-tumour activity of natural killer cells have shown that they exhibit more activity against undifferentiated tumour cells expressing "fetal-like" antigens than against their more differentiated counterparts.⁴⁶ Indeed, some workers believe that the cell is the effector cell in murine GvHD.^{47,48}

Effector cell population

As previously indicated, the exact nature of the effector cell population in human GvHD is unclear. Several workers have attempted to determine the pathogenic cell type in GvHD.

Many studies have shown that the predominant T cells in the lesional tissue of GvHD are CD8+ T cytotoxic (suppressor) cells.^{49,50} These data have been extrapolated to implicate CD8+ cells in the pathogenesis of GvHD and have formed the basis for suggestion that the CD8+ T cell population is the effector cell population in GvHD.^{51,52}

Tissue lymphoid repopulation, however, may merely reflect lymphoid reconstitution in the peripheral blood. Many workers have shown that marrow grafting is followed by a reversal in the peripheral CD4+:CD8+ ratio, which is normally 2:1.^{53,54} This reversal in the normal CD4+:CD8+ ratio occurs regardless of the presence or absence of GvHD.⁵⁵ The increase in the number of CD8+ cells in the period after transplantation seems to correlate merely with increasing time after transplantation and not with the development of GvHD.

Thus the predominance of CD8+ cells in tissues affected by GvHD may merely reflect the predominance of CD8+ cells in the peripheral blood, and not an active infiltration of CD8+ cells into the lesional tissue, and thus may have little bearing on the pathogenesis of GvHD.

Furthermore, several recent studies, particularly in animals, have highlighted the role of the CD4+ T cell in GvHD.^{56,57} Evidence from murine models⁵⁸ and from in vitro studies of alloantigen induced cytolytic T cell generation⁵⁹ have indicated that both CD4+ and CD8+ cells are required to induce GvHD. Antihist CD4+ cells may induce GvHD by the release of cytokines, or by triggering a delayed type (T cell mediated) hypersensitivity reaction.^{60,61}

The issue of a discrete effector cell population mediating the pathogenesis of GvHD is thus unclear. Both T cell subsets seem to play an important part in the mechanism of the GvHD effector pathway. It is clear that complex cellular and humoral interactions are involved.

Role of environmental factors

Although initial studies suggested that GvHD occurs only in the context of histocompatibility antigen disparity between donor-recipient pairs,⁶² more recent evidence has shown that GvHD can occur in the absence of HLA disparity. Histologically confirmed GvHD has been described in recipients of syngeneic and even autologous marrow.^{63,64} Some of these patients had peritransplant viral infections, suggesting that reactivity to environmental antigens may have a role in the pathogenesis of GvHD.^{65,66} Moreover, GvHD is clinically variable and unpredictable,^{67,68} suggesting that non-MHC antigens may be involved in its exacerbation or initiation.

Over the past decade an increasing number of studies have reported an association between certain viruses and the development of GvHD in recipients of marrow grafts from HLA-matched sibling donors.⁶⁹⁻⁷² The frequent clinical association of cytomegalovirus (CMV) infection with GvHD has focused particular attention on the herpes viruses, although little is known about the possible pathogenic interaction of these two processes.

Studies in animal models have shown that simultaneous infection with murine CMV and GvHR to MHC antigens seems to act synergistically to induce GvHD.⁷³⁻⁷⁵ Murine CMV infection alters the immune response to allogeneic and hapten-modified syngeneic histocompatibility antigens, suggesting that CMV infection can enhance the allogeneic response and result in GvHD.

Herpes viruses may augment the severity of GvHD by modulating the normal immune response of the host or by modifying the expression of histocompatibility antigens by host cells, thus altering their surface antigenicity and acting as a target for donor immune surveillance.⁷⁶

Attempts to link herpes virus infection and GvHD in a causal relationship have mainly been based on seroepidemiological studies. As determined by donor and recipient seropositivity and, more recently, by the antiviral cellular immune response of the donor, both CMV and herpes simplex virus have been linked to an increased incidence of severe minor antigen induced GvHD.^{77,78}

In a large multicentre study, seropositivity to CMV before bone marrow transplant was identified as the major risk factor for the development of moderate to severe GvHD in multivariate analysis.⁷⁹ Positive donor and recipient seropositivity to CMV was also a major risk factor for the development of chronic GvHD. However, no studies have attempted prospectively to analyse organs affected by GvHD for herpes virus infection.

Future directions

GvHD is a fascinating disease which presents a useful model for a variety of immunological diseases and which provides insights into the mechanisms of immunocompetence and its establishment.

Further research aimed at improving our

understanding of transplantation immunology in general, and the pathogenesis of GvHD in particular, is required. Bone marrow transplantation is emerging as an important treatment modality for an increasing range of life threatening diseases, but its wider application is limited by failure to resolve the most serious complication of allogeneic grafting. A greater understanding of the pathogenesis of GvHD would enable allogeneic transplantation to be offered to patients without HLA-matched sibling donors and would make safer the transplantation of marrow from mismatched and matched unrelated donors.

It may not be possible to eradicate GvHD, and, indeed, the loss of the beneficial graft versus leukaemia (GVL) effect associated with GvHD may not be desirable in some patients. Graft manipulation which results in eradication of GvHD is also associated with reduced marrow engraftment and increased rejection (host versus graft reaction).^{80,81} Improvements in the prevention and treatment of GvHD, however, are necessary.

Current investigations into the efficacy of immunomodulators such as monoclonal antibodies directed against individual cytokines and their cellular receptors are producing some promising results.⁸²⁻⁸⁴ Similarly, manipulation of graft effector cell activity through graft T cell depletion and the use of antithymocyte globulin (ATG) have proved of benefit,^{85,86} and as evidence emerges that the cells mediating GVL are at least partially distinguishable from those causing GvHD,⁸⁷ selective T cell depletion of allogeneic grafts is likely to become an increasingly successful technique.

A clearer understanding of the association between herpes viruses and GvHD also has implications for the management of bone marrow transplant recipients. Definitive evidence that herpes virus infection exacerbates GvHD would justify active prophylaxis with acyclovir, or passive prophylaxis with anti-CMV hyperimmune globulin, for all recipients, and would indicate clearly the need for increased viral surveillance, with effective and early treatment of viral infective episodes, in seropositive patients.

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