LEADING ARTICLE

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Intestinal Graft-Versus-Host Disease
Mechanisms and Management

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Abstract

Allogeneic haematopoietic stem cell transplantation remains the treatment of choice for a number of malignancies. However, graft-versus-host disease (GVHD) has long been regarded as a serious complication of this procedure. Although GVHD may affect any organ, intestinal GVHD is particularly important because of its frequency, severity and impact on the general condition of the patient.

Recent studies have led to progressive elucidation of the mechanism of GVHD. Donor T cells are critical for the induction of GVHD, because depletion of T cells from bone marrow grafts effectively prevents GVHD but also results in an increase of leukaemia relapse. It has been shown that the gastrointestinal tract plays a major role in the amplification of systemic disease because gastrointestinal damage increases the translocation of endotoxins, which promotes further inflammation and additional gastrointestinal damage. Consequently, the management of intestinal GVHD (and the intestine itself) is a subject that should be highlighted.

In this article, approaches to the prevention of intestinal GVHD are discussed after being classified into three categories: regimens in common clinical use, regimens under investigation and original regimens used at our hospital. The standard regimen that is used most widely for prevention of GVHD is cyclosporin plus short-term methotrexate. Corticosteroids can be added to this regimen but careful consideration of the adverse effects of these hormones should be considered. Tacrolimus is a newer, more potent alternative to cyclosporin. T-cell depletion (TCD) after transplantation has been shown to prevent acute GVHD, however, the survival benefit of TCD has not been as great as expected. Mycophenolate mofetil can be useful for the treatment of acute GVHD as part of combination therapy. Regimens currently under investigation in animal experiments include suppression of inflammatory cytokines and inhibition of T-cell activation, and, specifically at our institution, hepatocyte growth factor gene therapy. The evidence-based therapy used at our institution includes systemic antibacterial therapy (including eradication of intestinal bacteria) to prevent the intestinal translocation of lipopolysaccharide and avoid the subsequent increase of various inflammatory cytokines. In addition, because of the similarities between intestinal GVHD and ulcerative colitis, sulfasalazine, betamethasone enemas and eicosapentaenoic acid have been used to treat intestinal GVHD in some patients.

Recently, allogeneic stem cell transplantation (SCT) has been increasingly used to treat solid tumours,[1] as well as haematopoietic disorders and haematological malignancies.[2] The efficacy rate has been improving steadily because of recent technical progress but it is still not high enough because of various complications. Among the complications of SCT, graft-versus-host disease (GVHD) is one of the major causes of death.[3,4] Intestinal GVHD is one of the most frequent non-lymphoid features of acute GVHD in humans, occurring at the same time as or shortly after the onset of cutaneous GVHD. There is increasing experimental and clinical evidence that damage to the gastrointestinal (GI) tract plays a major role in the amplification of systemic disease after SCT.[5] This article discusses the mechanism and management of acute GVHD with emphasis on intestinal GVHD.

1. Mechanism

1.1 Acute Graft-Versus-Host Disease (GVHD)

GVHD is a pathological state that arises secondary to the engraftment of donor T lymphocytes, which recognise the recipient's tissues as foreign and then attack various organs.[6] Acute GVHD manifests with skin rash, diarrhoea, jaundice and wasting; so the predominant symptoms are related to the skin, liver and GI tract, and these organs show characteristic mononuclear cell infiltration and histopathologic damage. From the pathophysiological standpoint, the course of GVHD can be divided into the following three phases (figure 1): (i) damage and cellular activation induced by preconditioning of the patient; (ii) activation of donor lymphocytes (T cells); and (iii) development of GVHD (cytotoxicity).[5,7]

1.1.1 Phase 1: Damage and Cellular Activation Induced by Preconditioning

The hallmark of phase 1 is cell and tissue damage caused by preconditioning. Tissue damage also leads to cellular activation and the release of inflammatory cytokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6. These cytokines induce the up-regulation of host antigens and adhesion molecules, leading to a response of donor T cells. Damage to the GI mucosa induced by preconditioning also permits bacteria and bacterial toxins to enter the body from the GI tract.[5,8]

1.1.2 Phase 2: Activation of Donor Lymphocytes (T Cells)

Donor T cells recognise host antigens presented by antigen-presenting cells (APC) and treat the host tissue as foreign so that an immune reaction is induced. After recognising host antigens, donor T cells differentiate into Th1 cells that produce type 1 cytokines (IL-2 and interferon [IFN]-γ).[9]

1.1.3 Phase 3: Development of GVHD (Cytotoxicity)

Phase 3 is the stage when cells are injured. The Th1 cells that underwent differentiation in phase 2 induce cytotoxic T cells (CTL) through the Fas/FasL and perforin/granzyme B systems, and also activate natural killer (NK) cells. These cells eventually attack various host cells. Th1 cells can also prime macrophages, leading to increased production of inflammatory cytokines, such as TNFα or IL-1, and increased release of nitric oxide (NO) in response to stimulation by lipopolysaccharide (LPS), which enters the body from the GI tract. These cytotoxic molecules directly attack various host tissues and cause the clinical manifestations of GVHD.[10,11]

1.2 Intestinal GVHD

In the intestine, the Peyer's patches and the lamina propria contain many lymphocytes. There are also many specific intraepithelial lymphocytes among the epithelial cells of the bowel. This dense collection of lymphocytes in the intestine forms the gut-associated lymphoid tissue (GALT), which is independent of the systemic immune system and is a biological defence mechanism with its own important immunological role. The GALT is not only the largest immune compartment in the body (accounting for one-fourth of all immunocytes), but is also in intimate contact with a specialised epithelial layer that is essential for the survival of the host. Therefore, damage to the intestinal mucosa

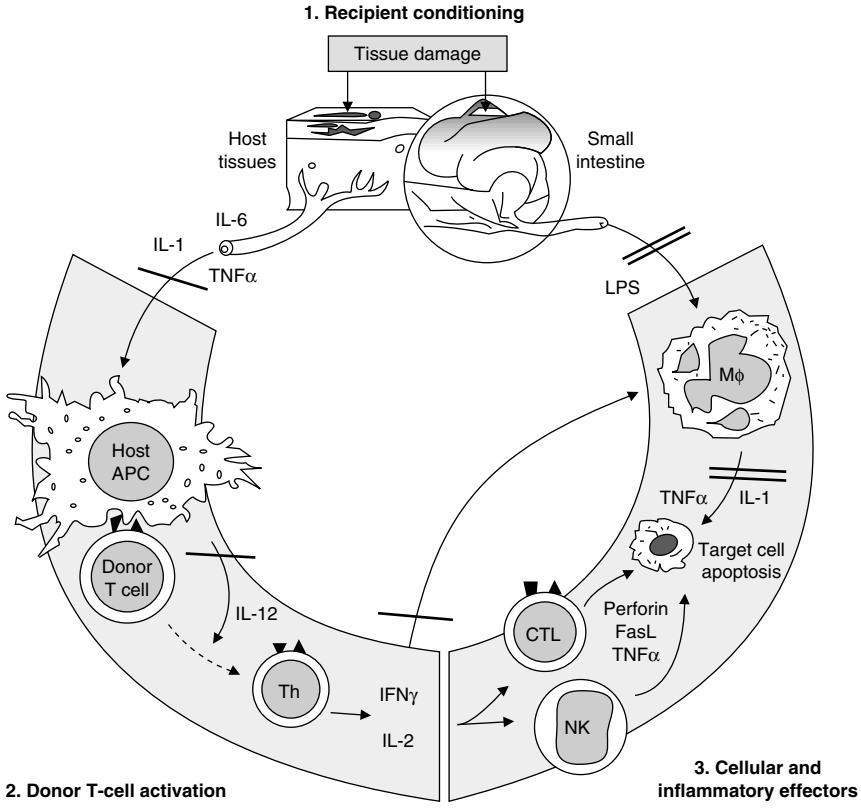


Fig. 1. The immunopathophysiology of graft-versus-host disease (reproduced with permission from Hill and Ferrara[5]). **APC** = antigen-presenting cell; **CTL** = cytotoxic T lymphocyte; **IFN** = interferon; **IL** = interleukin; **LPS** = lipopolysaccharide; **Mø** = macrophage; **NK** = natural killer (cell); **Th** = T helper cell; **TNF** = tumour necrosis factor.

may induce an inflammatory response that can induce or amplify the cytokine cascade involved in GVHD.

There have been several reports that the pathophysiological mechanism causing aggravation of GVHD involves intestinal damage related to preconditioning.[8] Bacterial endotoxin or LPS is produced by normal bowel flora and is a potent stimulator of the production of inflammatory cytokines, such as TNFα, IL-1 and IL-12. Damage to the GI tract by preconditioning allows the translocation of LPS into the systemic circulation. After the onset of GVHD, IFNγ produced by donor Th1 cells renders macrophages extremely sensitive to exogenous LPS. Thus, more extensive damage to the GI tract triggers higher systemic levels of inflammatory cytokines and leads to more severe GVHD.[12-16]

Although the Fas/FasL and perforin/granzyme B systems, as well as inflammatory cytokines, are involved in the mechanism of GVHD, intestinal GVHD has been mostly associated with TNFα (produced by donor T cells or other effector cells, such as macrophages) in mouse and human studies.[12,15,17] Expression of FasL by intraepithelial lymphocytes is up-regulated during GVHD and these cells can induce Fas-dependent intestinal GVHD on transfer to normal mice.[18] However, in contrast to anti-TNFα antibodies, administration of neutralising anti-FasL antibodies or the transfer of FasL-deficient donor T cells has shown no ef-

fect on the development of intestinal GVHD in several studies.[17,19,20]

2. Management

There are various approaches to the management of intestinal GVHD that have been divided into the following four categories for description.

- Regimens that are widely used at present.
- Regimens that are under investigation.
- Original regimens that are used at our hospital.
- Regimens that will be used or tested in animal experiments at our institution in the future.

2.1 Regimens That Are Widely Used at Present

2.1.1 Cyclosporin

In the 1980s, cyclosporin became available clinically and this drug has since been employed in various regimens (table I). The standard regimen that is used most widely for prevention of GVHD is cyclosporin plus short-term methotrexate.[21] Cyclosporin is a metabolite of two species of fungi that were isolated from soil samples collected in the Hardanger highlands of Norway in 1970.[22,23] It is a cyclic peptide consisting of 11 amino acid residues (figure 2) and its immunosuppressive mechanism is based on an action against calcineurin.[24] Cyclosporin forms a complex with cyclophilin. This complex inhibits the dephosphorylase activity of activated calcineurin and thereby inhibits translocation of cytoplasmic nuclear factor of activated T cells (NFAT) into the nucleus, leading to suppression of the transcription of IL-2 mRNA and also blocking the expression of many other cytokines, thus causing immunosuppression.[25-27] After HLA-compatible stem cell transplantation from a sibling donor, cyclosporin plus short-term methotrexate is an international standard regimen for the prevention of GVHD in adult patients.[28]

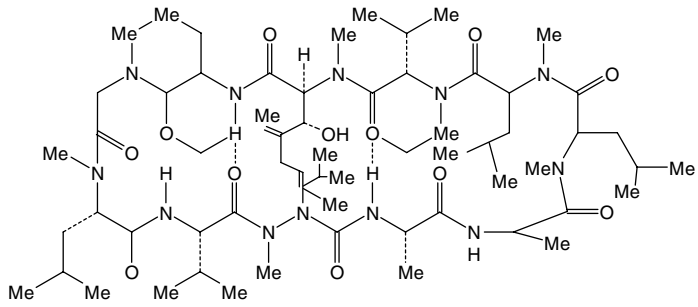
2.1.2 Corticosteroids

GVHD prophylaxis with a three-drug combination consisting of cyclosporin, methotrexate and methylprednisolone was reported by Chao et al. in 1993.[31] At the cellular level, the immunosuppressant effects of corticosteroid therapy have not yet been fully elucidated but the actions mediated by intracellular steroid receptors (GR) are considered central.[34] Steroid-GR complexes bind to each other and form dimers after migration into the nucleus. These dimers are considered to act as a transcription factor that binds to the binding site of the glucocorticoid receptor (CRE) on DNA, and thus blocks the expression of various cytokines and enzymes.[35,36] In addition, it suppresses transcription factors, such as activator protein (AP)-1 (Jun/Fas complex) and nuclear factor (NF)κB,[37-39] and hence inhibits the expression of molecules that are induced by these factors, including cytokines, various enzymes and receptors. Because corticosteroids are hormones, these agents have various actions and cause numerous adverse effects on systemic metabolism, the cardiovascular system, the gastrointestinal tract, the central nervous system and bone. Whenever corticosteroids are pre-

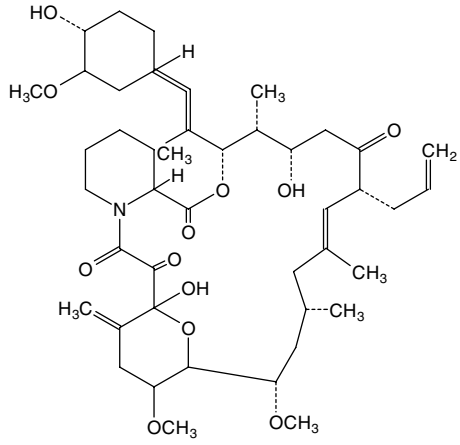
Table I. Randomised studies on the prevention of graft-versus-host disease after stem cell transplantation from HLA-matched sibling donors

Reference	Disease	Median age (y)	No. of patients	Prophylaxis	Survival rate (%)
Forman et al.[29]	AL, CML	26	54	CsA/P	57
Santos et al.[30]	Leukaemia	23	42	CsA/mP	38
Chao et al.[31]	Malignancy	32	74	CsA/P	59
			60	CsA	18
Deeg et al.[32]	Malignancy		62	CsA/mP	22
			28	l-CsA	68
Zikos et al.[33]	AML		32	l-CsA/l-MTX	74

AL = acute leukaemia; **AML** = acute myelogenous leukaemia; **CML** = chronic myelogenous leukaemia; **CsA** = cyclosporin; **P** = prednisolone; **mP** = methylprednisolone; **l-CsA** = low-dose CsA; **l-MTX** = low-dose methotrexate.



Cyclosporin



Tacrolimus

Fig. 2. Chemical structures of cyclosporin and tacrolimus (FK-506).

scribed, these adverse effects should be carefully considered.

2.1.3 Tacrolimus

In 1984, tacrolimus (FK-506) was detected as a metabolite of *Actinomyces* spp. that was isolated from a soil sample collected in Tsukuba (Ibaragi Prefecture, Japan).[40] This drug is far more effective than cyclosporin. In 1989, Starzl reported that it could suppress ongoing rejection after liver transplantation when cyclosporin had proved ineffective[41](figure 2). In 1990, it was confirmed by clinical studies that tacrolimus was effective and could be safely used, and it became generally available for clinical use. Like cyclosporin, tacrolimus forms a complex with a specific protein (FK506-binding protein [FKBP]), and this complex binds to calcineurin and inhibits its dephosphorylase activity.[24] As a consequence, the migration of NFAT (the IL-2 gene transcription factor) into the nucleus and the subsequent biosynthesis of IL-2 are prevented, giving rise to immunosuppression.[27,42] Tacrolimus is 50–100 times more potent than cyclosporin on a milligram-per-milligram basis.

Ratanatharathorn et al.[43] and Nash et al.,[44] respectively, reported that tacrolimus reduced the incidence of acute GVHD after bone marrow transplant (BMT) from HLA-matched siblings and

HLA-mismatched unrelated donors. The incidence of grade II–IV acute GVHD was significantly lower in patients who received tacrolimus than in patients treated with cyclosporin (31.9 vs 44.4%, respectively; p = 0.01). The incidence of grade III–IV acute GVHD was similar (13.3 vs 17.1%, respectively). There was no difference in the incidence of chronic GVHD between the tacrolimus and cyclosporin groups (55.9 vs 49.4%, respectively; p = 0.8). According to Hiraoka et al.,[45] a controlled clinical trial showed that the incidence of grade II–IV acute GVHD within 100 days after transplantation was significantly lower among patients receiving tacrolimus than among those receiving cyclosporin (p < 0.0001)[figure 3a], but the recurrence rate after transplantation from HLA-matched sibling donors was significantly higher in patients treated with tacrolimus (p = 0.0013) [figure 3b].

Consequently, the GVHD prevention regimen should be prescribed in consideration of both the graft-versus-leukaemia (GVL) effect and the type of donor. In addition, tacrolimus causes adverse effects such as renal dysfunction, neurological dysfunction, hyperglycaemia and hypertension. Because the incidence of these adverse effects increases in proportion to the trough plasma concentration of the drug, frequent measurement of the plasma concentration is necessary so that the dose can be reduced promptly when necessary.[46]

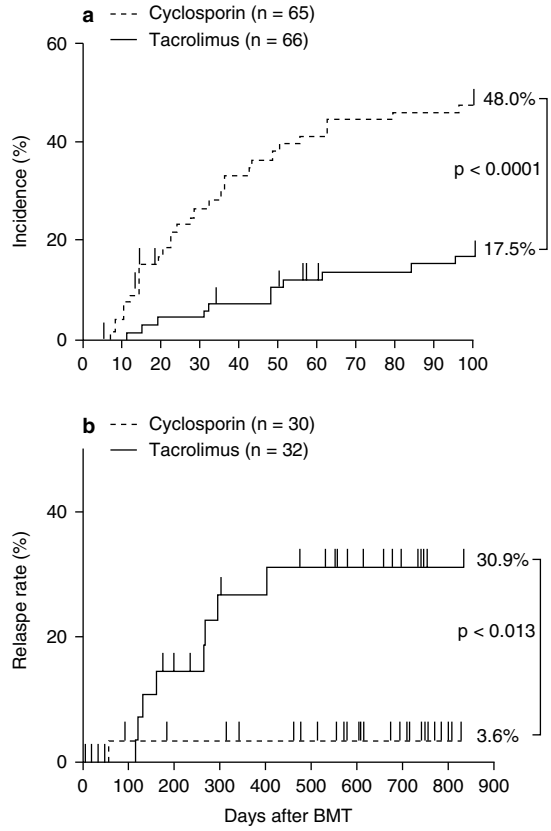


Fig. 3. Cumulative incidence of grade II-IV acute graft-versus-host disease in patients receiving tacrolimus versus those receiving cyclosporin during the initial 100-day period after bone marrow transplantation (BMT) (**a**) and relapse rate at final observation in recipients from HLA-matched siblings from the same controlled clinical trial (**b**) [reproduced with permission from Hiraoka et al.[45]].

2.1.4 T-Cell Depletion

There have been many reports that the development of acute GVHD can be prevented by depletion of T cells (TCD) after transplantation from not only HLA-matched sibling donors but also HLA-mismatched donors.[47-51] Despite the prevention of acute GVHD, the survival benefit of TCD has not been as great as expected because of an increased rejection rate, an increased post-transplantation recurrence rate and an increased incidence of infection due to delayed recovery of immunity. TCD has often been used in the case of SCT from HLA-matched sibling donors to treat acute myelogenous leukaemia (AML) or acute lymphoblastic leukaemia (ALL). Typically, Soiffer et al. employed CD6 antibodies for TCD,[52] while Bunjes et al. used campath-1.[53] The patients treated in these two studies had only achieved complete remission once before transplantation and did not receive any other immunosuppressants. They reported that the rejection rate and the incidence of grade III or higher acute GVHD were decreased, with few deaths related to transplantation.

After SCT from unrelated donors, Drobyski et al. performed TCD with T cell receptor antibodies[54]and Oakhill et al. used campath-1.[55] The re-

jection rates for patients treated with TCD were comparable to those for patients without TCD, but the incidence of grade III or higher GVHD was only about 7% in the former group and was lower than in the patients without TCD.

2.1.5 Mycophenolate Mofetil

Mycophenolate mofetil (MMF), also known as RS-61443, is an ester of mycophenolic acid that inhibits the *de novo* synthesis of guanine nucleotides.[56] After oral administration MMF is hydrolysed by esterase in the intestine and blood to release mycophenolic acid (MPA). MPA has a more potent cytostatic effect on lymphocytes than on other cells and this is the principal mechanism of its immunosuppressive activity.[57] MMF is useful for the treatment of GVHD in combination with cyclosporin and prednisolone.[58]

2.2 Regimens Under Investigation in Animal Experiments

2.2.1 Suppression of Inflammatory Cytokines

As described in the section on the mechanism of GVHD (section 1), the intestine is a target organ for GVHD and it simultaneously augments GVHD by stimulating the production of TNFα due to the translocation of LPS.[6] It is possible that GVHD could be alleviated if agents were developed that could protect the intestinal mucosa, reduce the translocation of LPS and reduce the production of TNFα. GVHD develops via a complex process with several stages that involves many factors. Consequently, there have been numerous failed attempts to prevent GVHD by using antibodies and receptor antagonists.[59]

Hill and colleagues examined the effect of recombinant human IL-11 and keratinocyte growth factor (KGF) on experimental acute GVHD. These two cytokines effectively prevented intestinal injury caused by acute GVHD, thereby inhibiting the translocation of LPS from the gut lumen into the systemic circulation and subsequent production of inflammatory cytokines.[60-62] Recently, Cooke et al. took a more direct approach to blocking GVHD. They showed that B975, a direct competitive antagonist of endotoxin, attenuated the inflammatory response and improved acute GVHD.[63] These approaches preserved the responses of donor T cells to host antigens and promoted leukaemia-free survival after BMT by reducing acute GVHD while preserving GVL activity.[61-63]

However, GVHD in humans is a complex process that is unlikely to be controlled by a single agent. Most studies show that some benefit can be obtained through various approaches, including cytokine inhibition and IL-11 administration.[15,64] A useful strategy may be to attempt GVHD control by recognising the underlying pathophysiology and interfering with different steps along the pathway by using several approaches in combination. Recently, promising results were reported in the case of steroid-resistant acute or chronic GVHD. Infliximab, a chimeric human/mouse anti-TNFα antibody, significantly improved steroid-resistant acute GVHD,[65] while etanercept (recombinant soluble TNFα receptor) improved chronic GVHD and allowed tapering of the corticosteroid dosage.[66]

2.2.2 Inhibition of T-Cell Activation

T cells are activated after the induction of IL-2 production[67] and IL-2 receptor (IL-2R) expression.[68] The occurrence of these two events requires T-cell receptor (TCR) signalling (the first signal) which is induced by stimulation with an antigen+MHC (major histocompatibility complex) on an APC, as well an additional second signal or supplementary stimulation with CD28.[69-74]

Therefore, prevention of GVHD can be based on the inhibition of T-cell activation by blocking TCR signalling and/or supplementary stimulation by CD28. BC3 is a monoclonal antibody that reacts with the CD3 complex, but is non-mitogenic as a result of inefficient interaction with the Fc receptor on human monocytes; it was reported to improve clinical manifestations of GVHD.[75] CTL-associated antigen (CTLA)-4, which binds to CD80/CD86 on APC far more strongly than CD28,[76] has been used to produce a chimera protein (CTLA-4Ig) that blocks supplementary stimulation with CD28, and the engraftment of heterogeneic cells was successfully prolonged using this agent.[77,78] The use of

an anti-IL-2 receptor monoclonal antibody (daclizumab) to treat patients with steroid-resistant GVHD has also been reported.[79] The complete response rates in two clinical studies were 29 and 47%, respectively.

2.3 Original Regimens Used at Our Institution

The evidence-based therapy in general use at our institution is described in this section. We have found that the underlying mechanism of post-transplantation complications including GVHD is vascular endothelial injury, the development of which is mediated by an increase of inflammatory cytokines that is stimulated by infection and other factors.[80-88] We have called this process the systemic inflammatory response syndrome (SIRS) and have reported our hypothesis that it plays an aetiologic role in posttransplantation complications.[89,90] In addition to SIRS, another mechanism called the 'second attack' theory[90]may be involved in systemic symptoms.[89] This theory suggests that cells are primed to produce inflammatory cytokines by a certain stimulus (the first attack) and then cytokines are released in response to another stimulus (the second attack), leading to the aggravation of systemic symptoms[91](figure 4).

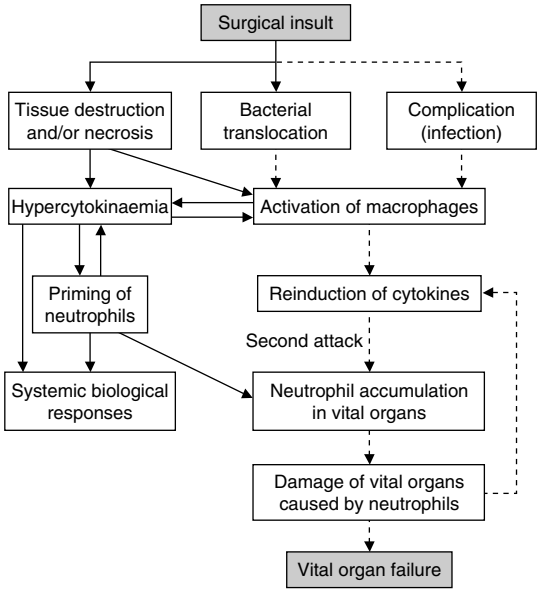


Fig. 4. Mechanisms of the development of organ failure after a surgical insult: the 'second attack' theory (reproduced with permission from Ogawa.[91])

On the basis of these findings, systemic antibacterial therapy (including eradication of intestinal bacteria) is used at our institution to prevent GVHD, particularly intestinal GVHD. This therapy aims to inhibit both the first and second attacks of the 'second attack' theory described above by preventing the intestinal translocation of LPS and avoiding the subsequent increase of various inflammatory cytokines, including TNFα. It has been demonstrated that intestinal GVHD and ulcerative colitis share the same cytokine profile, with a characteristic increase of IL-7, as well as sharing various endoscopic and histological features.[92-94] Similarly, leukotriene (LT)B_4 is a risk factor for ulcerative colitis and it also predicts the onset of intestinal GVHD.[95-97] Because of such similarities between these conditions, the following agents for ulcerative colitis therapy have been used to treat intestinal GVHD in some patients.

2.3.1 Sulfasalazine

Among the drugs used for the treatment of ulcerative colitis, the effect of sulfasalazine (SASP) on intestinal GVHD is described first. SASP is split into 5-aminosalicylic acid (5-ASA) and sulfapyridine in the large intestine. 5-ASA has been shown to inhibit a variety of inflammatory and immunological processes, and to impair neutrophil function. This drug has long been used for the treatment of inflammatory bowel disease and arthritis. According to Wanders et al., SASP increased the effectiveness of low-dose cyclosporin in preventing rejection after heart transplantation in rats.[98] Prevention of allograft rejection was considered to be partly based on the inhibition of IL-2 production by SASP, as was the case for cyclosporin. SASP can down-regulate the immune response by a mechanism which seems to be distinct from that of cyclosporin. A patient for whom SASP was effective at our institution is described in figure 5.[99]

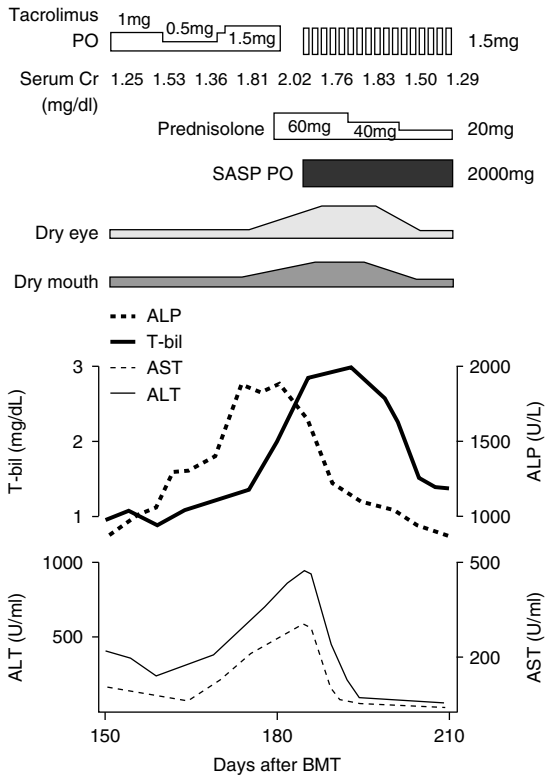


Fig. 5. A 42-year-old man with chronic myeloid leukaemia (CML), who received bone marrow transplantation from a sibling donor. For prevention of GVHD, he was treated with CsA and short-term MTX. After transplantation, his renal function decreased along with aggravation of GVHD. Consequently, the dose of CsA was changed and then tacrolimus was administered instead of CsA, while prednisolone and methylprednisolone were added in an attempt to control GVHD. However, all of these measures failed. On day 187, SASP was started at a dose of 2 000 mg/day. Subsequently, his intestinal and hepatic GVHD were controlled (reproduced from Okada[99] with permission from Karger, Basel). **ALP** = alkaline phosphatase; **ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase; **Cr** = creatinine; **CsA** = cyclosporin; **GVHD** = graft-versus-host-disease; **MTX** = methotrexate; **PO** = orally; **SASP** = sulfasalazine; **T-bil** = total bilirubin.

2.3.2 Betamethasone Enemas

The second regimen is betamethasone enemas. Betamethasone is a corticosteroid that was originally used to treat inflammatory bowel disease, particularly ulcerative colitis. Because this therapy is not systemic a high dose can be delivered to the affected site, so it has a potent effect with few adverse reactions. We have found that betamethasone enemas achieve a good response within about 3 weeks in many patients with severe intestinal GVHD showing resistance to standard GVHD prophylaxis. The stage of GVHD and the details of betamethasone enema therapy are summarised in table II. The dosage of cyclosporin was not increased during enema therapy and betamethasone enemas were administered for 5 to 27 days. In six of the eight patients, diarrhoea/or abdominal pain resolved. The seventh patient was treated for 18 days but no improvement of symptoms was observed, while the eighth patient was not able to tolerate enema therapy because of a poor general condition. These latter two patients died of thrombotic thrombocytopenic purpura and idiopathic pneumonitis, respectively. In the responding patients, intestinal GVHD did not worsen after enema therapy was discontinued and it was possible to reduce the dosage of systemic corticosteroids without recurrence of intestinal GVHD. The enemas were well tolerated by all patients other than the eighth patient and no severe adverse effects were observed.[100]

2.3.3 Eicosapentaenoic Acid

The third treatment is eicosapentaenoic acid (EPA), an ω-3 polyunsaturated fatty acid. It was originally used as a antithrombotic and antiatherosclerotic drug,[101,102] but it also inhibits inflammatory cytokines and reduces vascular endothelial damage due to SIRS.[103-108] Consequently, it is not only effective against all post-transplantation complications, but can also ameliorate intestinal GVHD by reducing LTB_4 production and hence decreasing various adverse effects of white blood cells on the intestine. Arachidonic acid in the membranes of white blood cells is metabolised to LTB_4, which promotes the migration, adhesion and aggregation of leucocytes. LTB_4 also has various potent pro-inflammatory actions, including the release of lysosome, activation of natural killer cells, and promotion of the production of TNFα, IL-2 and IFNγ. Like arachidonic acid, EPA is metabolised by 5-lipoxygenase,

Table II. The stage of graft-versus-host disease (GVHD) and details of betamethasone enema therapy[100]

Patient	Stage of acute GVHD			Onset of intestinal GVHD (day)[a]	Betamethasone enema		
	Skin	Liver	Intestine		Duration (days)	Start (day)[a]	Response (stage)
1	I	None	II	80	116	20	II→0
2	I	I	III	20	40	12	III→0
3	None	None	IV	29	31	18	IV→0
4	None	None	III	165	175	27	III→0
5	None	None	II	100	133	21	II→0
6	II	I	III	44	46	10	III→0
7	I	None	IV	25	29	18	IV→IV
8	I	None	III	17	18	5	Not evaluable

a 'day' indicates the day after transplantation.

but it forms LTB_5 instead of LTB_4. Although LTB_5 also binds to the LTB_4 receptor and also activates leucocytes to promote the development of inflammation, it causes far less damage than LTB_4. Inhibition of platelet aggregation by EPA is related to its prevention of vascular endothelial dysfunction. Namely, EPA acts on platelets as well as endothelial cells and smooth muscle cells in the vessel wall, by replacing arachidonic acid in the cell membrane. When EPA is metabolised by cyclooxygenase in platelets and the vascular wall, it forms thromboxane $(TXA)_3$ and prostaglandin $(PG)I_3$, respectively. Unlike TXA_2, TXA_3 does not enhance platelet aggregation, while PGI_3 is as effective as PGI_2 in inhibiting platelet aggregation. Thus, the net effect of EPA is to cause the inhibition of platelet aggregation.[109,110]

2.4 Hepatocyte Growth Factor Gene Therapy

The final therapy to mention is hepatocyte growth factor (HGF), which is currently under investigation in animal experiments at this institution. HGF was originally identified and cloned as a potent mitogen for hepatocytes.[111,112] It also has mitogenic, motogenic and morphogenic effects on various nonhepatic epithelial tissues, particularly the kidneys, lungs and intestine.[113-115] Furthermore, HGF shows anti-apoptotic activity[116] and plays a role in enhancing haematopoiesis.[117] Intravenous injection of recombinant human HGF has been shown to enhance liver and kidney regeneration in mice, as well as preventing acute renal failure and suppressing the onset of liver cirrhosis induced by dimethylnitrosamine,[113,118] suggesting that HGF plays an important role in the tissue repair process.

We recently demonstrated that serum HGF levels were significantly increased in patients with acute GVHD, suggesting that HGF was produced to counteract tissue damage caused by GVHD.[119] Therefore, we attempted to treat acute GVHD with HGF in a well-characterised mouse model of this disease. We used the transgene approach instead of a recombinant protein for the following reasons: (i) the half-life of the recombinant protein is quite short, so massive doses of active HGF would need to be given frequently; (ii) high doses of active HGF protein may cause adverse effects such as tumorigenesis in other organs;[120] and (iii) recombinant HGF is very costly. In contrast, the transgene approach is simple, safer, cheap and needs much less frequent administration. Repeated transfection of the human HGF gene into skeletal muscle in our mouse model of GVHD promoted haematopoietic function, and strongly inhibited acute GVHD by limiting tissue damage and the subsequent endotoxin-mediated inflammatory cascade.[121] HGF prevented GI injury by an anti-apoptotic effect on intestinal epithelial cells and thus blocked the endotoxin-mediated inflammatory cascade. Inhibition of the translocation of endotoxin by prevention of GI injury through HGF treatment also

resulted in the inhibition of donor T-cell expansion in the liver, thereby ameliorating the hepatic injury caused by GVHD.

This approach to the treatment of GVHD aims to reduce tissue damage and the subsequent inflammatory immune response. It may be possible to overcome barriers to successful transplantation from major-HLA mismatched donors by using this approach in combination with other methods such as immunosuppression or inhibition of cytokine production.

3. Differential Diagnosis

Finally, it is important to describe the diseases from which GVHD should be distinguished. The differential diagnosis includes three conditions, which are regimen-related toxicity (RRT), infection and ischaemic enteritis as a result of thrombotic microangiopathy (TMA). RRT is prevalent among high-risk patients with haematological malignancies and those receiving intensified preconditioning. Because RRT subsides within 3–4 weeks after transplantation, only infection and ischaemic enteritis need to be considered after this initial period.

Among infections that cause diarrhoea, viral infection is the central condition to be detected. Cytomegalovirus (CMV), adenovirus, rotavirus and herpes simplex virus (HSV-1) have been reported as viruses that can infect the intestine, but CMV is the most important in this patient group. Because CMV infection is characterised by the formation of giant cells with inclusion bodies, histological examination is useful. Ischaemic enteritis as a result of TMA is a complication that has been reported recently. Diagnosis is not easy while the patient is alive, but TMA can be confirmed by the histological demonstration of platelet hyaline thrombi and fibrinoid necrosis of terminal arterioles in association with fragmentation of erythrocytes. At autopsy, thrombotic necrosis of submucosal arterioles is a common finding. CMV enteritis is curable, so ischaemic enteritis as a result of TMA has become the most important complication because it can cause severe bleeding from the lower GI tract.

Endoscopic evaluation, with histological examination of biopsy specimens, can be useful for diagnosing intestinal GVHD.[122] However, it is important to remember that both endoscopic evaluation and histology can underestimate the severity of intestinal GVHD.[123] Recently, non-invasive methods have been used to assess the extent and severity of intestinal GVHD, including computed tomography[124]and high-resolution ultrasonography.[125] These methods may become helpful tools for the diagnosis of intestinal GVHD in the future.

4. Conclusion

Recently, the mechanism of GVHD has been progressively elucidated and new treatments have been developed that have contributed to the control of intestinal GVHD. However, many approaches to the control of GVHD such as treatment with immunosuppressants or *ex vivo* T-cell depletion of the haematopoietic stem cell graft, reduce GVL activity because these approaches inhibit the donor T-cell response to host antigens. In the future, treatments need to be developed that can inhibit GVHD without decreasing the GVL effect. To achieve such ideal therapy, management of intestinal GVHD using cytokines like IL-11, KGF and HGF may be useful.

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