

Pathogenesis of Graft-versus-Host-Disease

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 • Cytokines

The diverse models in which graft-versus-host disease (GVHD) has been described range from *in vitro* cell preparations to whole animals and, of course, to clinical experience in human beings. However, the mechanisms of the disease and the manipulation of this reaction in favor of the patient toward a graft-versus-tumor effect have been persistent puzzles that are still undergoing intensive investigation.

The difficult, halting, and sometimes speculative task of a pathologist in both clinical and animal *in vivo* settings is to sort out this voluminous and often bewildering background of immunologic data to apply it to the interpretation of histopathologic specimens. The hope is that clinically useful conclusions can be drawn about diagnosis and disease progression and that some insights into pathogenesis may result. Because inflammatory diseases in all organs have a limited histologic expression, pathognomonic lesions are not claimed. The diagnosis remains a syndromic clinicopathologic one, even with histologic data available.

The allogeneic GVHD is defined as an allograft reaction of donor lymphoid cells against host histocompatibility antigens, minor or major. The classic basic requirement is the engraftment of an allogeneic lymphoid cell population. The mechanism, which involves donor T cells, cytokines, antigen-presenting cells, and cytotoxic lymphocytes inducing damage to target epithelial cells, is highly complex and controversial. The key goal, which has not yet been achieved, would be a synthetic integration into a coherent tolerance model.

I chose 3 of the animal models to illustrate pathogenetic points, with the realization that the clinical situation is more complicated. The cleanest *in vivo* model is the parent into F1 hybrid model, in which the genetics allow engraftment of the donor marrow

without rejection, but the donor lymphocytes can recognize the host as foreign. Hakim [1] cites the chronic GVHD model as requiring class II disparity and CD4 cell engraftment; this produces a T-helper type 2 pattern of cytokine production, with interleukin (IL)-4, IL-5, and IL-10 predominance (Table 1). Low donor chimerism is produced, as is lymphoid hyperplasia with autoimmune-like phenomena, including glomerulonephritis. This is unlike the human situation. The acute pattern requires CD4 and CD8 cells, as well as both class I and II disparity. This yields extensive chimerism, replacing the host marrow and lymphoid tissue and inducing a T-helper type 1 cytokine pattern with IL-2 and interferon- γ , and it induces cytotoxic lymphocytes to attack epithelial cells. This is similar to human acute GVHD. Over time, some of these animals develop a late phase similar to human chronic GVHD with lymphoid hyperplasia and periductal lesions of the liver and salivary glands.

The syngeneic or autologous GVHD mouse model is a special case in which true autoimmunity develops [2]. This requires an initially intact thymus, cyclosporine administration, and peripheral lymphoid damage, such as irradiation. The result is the development of autotoxic CD8⁺ lymphocytes that react against autologous class II antigens. A similar syndrome is seen in a small fraction of human autograft recipients.

The mouse model most closely resembling human marrow transplantation is the model of Korngold and Sprent as used by Hamilton and Parkman [3], in which minor histoincompatible pairs are transplanted after host irradiation. This produces a syndrome resembling human acute and chronic GVHD. The human situation is most analogous to this, but of course it is more complex, and the syndromes differ in detail somewhat [4,5].

Table 1. Differences among Chronic GVHR, Initial Phases of Acute GVHR, and Long-Term Acute GVHR (After Hakim [1]; Parent to F1 Hybrid Model)

Variable	Chronic GVHR	Acute GVHR Initial Phase	Acute GVHR Long Term
Donor effectors	CD4	CD4, CD8, NK	ND (CD4 and CD8 in humans)
Donor chimerism	4%-9%	95%	95%
Donor cytokines	IL-4, IL-10	IL-2, IFN- γ , TNF	IL-4
Autoantibodies	Yes	No	ND (yes in fraction of humans)
Thymic alterations	No	Yes	No (yes in humans)
Suppression of B- and T-cell responses	No	Yes	No (yes in humans)

GVHR, graft-versus-host reaction; NK, natural killer; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; ND, data not available.

The end result in human allograft recipients from a clinical and histopathologic point of view is cell death in the target epithelium (basal layer epithelial damage, or apoptosis) in the skin, lip, and gut, as well as the duct cells in the salivary glands and liver. The underlying damage to the lymph nodes and spleen, often called the *lymphoid suicide reaction* (perhaps better termed a fratricide) is basically a subclinical phenomenon, which in the allogeneic patient persists for many months. The autologous patient, however, recovers very quickly from the lymphoid and splenic damage. Acute GVHD is a clinical triad of dermatitis gastroenteritis and hepatitis that occurs roughly within the first 50 to 80 days after transplantation and is only easily diagnosed after chemotherapy and radiation damage, neutropenia, and infections have recovered. In the skin, epidermal basal cell damage with lymphocytic infiltration, spongiosis, and apoptosis are the characteristics of GVHD. Simultaneously, the perifollicular bulge of the hair follicle is damaged, and similar lesions are seen in that structure. Sometimes early, but usually later, sweat ducts are also attacked and damaged; the combined effects produce atrophy of most of the adnexa of the skin, especially in chronic GVHD. The lip biopsy sample, which also has a squamous mucosa, has a similar infiltration of mononuclear cells into the basal layer with destruction of the squamous epithelium on the surface. The minor salivary glands are usually involved simultaneously with the surface epithelium, particularly the ducts of the minor salivary glands, with the production of duct cell apoptosis, ectasia, fibrosis, and atrophy. In most of these sites, CD45⁺ lymphocytes with initially a CD4 and later a CD8 phenotype are easily identified on immunohistology. Later in the course of GVHD, either de novo or continuing from the acute disease, sclerodermatous lesions may develop with deep sclerosis of the dermis, atrophy of the epidermis, complete loss of the adnexa, and fasciitis, with damage to the subcutaneous fat and the production of a sclerodermatous skin similar to that after eosinophilic fasciitis. It is thought that autologous reactivity to the patient's own class II antigens may be active in this situation. The esophagus, which is also a squamous organ, is infiltrated similarly to the skin and lip with

infiltrating lymphocytes and apoptotic cells. The small intercalated bile ducts of the liver are prime targets in GVHD in most species examined, including mice, humans, horses, dogs, and nonhuman primates. This may be a result of the oval cell stem localization around the small bile ducts in the liver. The gastrointestinal tract, which is usually the cause of death in acute GVHD, is attacked in stem cell regions of the crypts near the necks of crypts with apoptotic cells, which may be present in single or multiple examples with lymphocytes and occasional eosinophils. Crypt damage, crypt loss, and even denudation of the epithelium with fatal bleeding or sepsis may follow. In the lung, acute GVHD remains controversial, but chronic GVHD clearly shows bronchiolitis obliterans and an association with bronchiolitis obliterans organizing pneumonia. Chronic GVHD may also show some infrequent manifestations such as myositis, serositis, arthritis, and autoantibodies. One of the key manifestations of chronic GVHD is, in addition to dry mouth, dry eyes, with an attack on the lacrimal glands and corneal epithelium identical to the lesions seen in salivary glands. These lesions can produce severe keratoconjunctivitis sicca and induce immune deficiency, thus allowing various conjunctival and scleral infections, especially herpes simplex. Emphasis in this talk will be placed on the hypothesis that the epithelial stem cell domains in the affected organs in GVHD comprise a common target region because of the presence of the proliferation of transient amplifying cells in the stem cell regions, such as the epidermal rete ridges, the perifollicular bulges, the crypts of the gut, the small bile ducts of the liver, and the filiform papillae of the tongue [6].

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