Overview

Miniature Swine as a Clinically Relevant Model of Graft-Versus-Host Disease

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Miniature swine provide a preclinical model of hematopoietic cell transplantation (HCT) for studies of graft-versus-host disease. HCT between MHC-matched or -mismatched pigs can be performed to mimic clinical scenarios with outcomes that closely resemble those observed in human HCT recipients. With myeloablative conditioning, HCT across MHC barriers is typically fatal, with pigs developing severe (grade III or IV) GVHD involving the gastrointestinal tract, liver, and skin. Unlike rodent models, miniature swine provide an opportunity to perform extended longitudinal studies on individual animals, because multiple tissue biopsies can be harvested without the need for euthanasia. In addition, we have developed a swine GVHD scoring system that parallels that used in the human clinical setting. Given the similarities of GVHD in pigs and humans, we hope that the use of this scoring system facilitates clinical and scientific discourse between the laboratory and the clinic. We anticipate that results of swine studies will support the development of new strategies to improve the identification and treatment of GVHD in clinical HCT scenarios.

Abbreviations: BMT, bone marrow transplantation; GVHD, graft-versus-host disease; GVL, graft-versus-leukemia; HCT, hematopoietic cell transplantation; TBI, total-body irradiation.

Clinical Significance

Many hematologic malignancies can be treated successfully with nonmyeloablative conditioning (Figure 1) followed by allogeneic (where donor and host are genetically disparate) bone-marrow transplantation (BMT). This treatment relies on the graft-versus-leukemia (GVL) reaction where donor T cells kill host tumor cells expressing either host or tumor associated antigens. The major drawback of the GVL effect, however, is graft-versus-host disease (GVHD), a life-threatening complication[19] of allogeneic hematopoietic cell transplantation (HCT).

The pig is an excellent animal model for the study of human diseases.[46] The Massachusetts General Hospital (MGH) miniature swine has been used extensively for the development of novel protocols related to BMT and cytokine-mobilized HCT (Figure 2 A and B). Other applications of swine HCT include studies on transplantation-associated complications, the development of novel peripheral-blood mobilization strategies, and the study of neoplastic disorders.[9,19]

GVHD

Acute GVHD involves the trafficking of donor T cells to specific areas of the body, namely the skin, intestine, and liver, and the subsequent attack on host cells in these locations.[90] GVHD can be summarized as a 3-step process. The first phase includes the conditioning regimen prior to transplantation, which causes damage to host tissues and induces activation of cells within these tissues. The intestine is affected in particular,[13,14] and the translocation of bacteria and bacterial toxins, such as LPS, is common. Donor T-cell activation occurs during the second phase when they encounter antigens expressed by host antigen-presenting cells. Polarization toward a Th1 response and secretion of cytokines, such as IL2, IL1 and IFNγ, occurs.[23] The effector (killer) functions of the activated lymphocytes occur during the third phase. Activated macrophages resulting from the conditioning regimen and cytotoxic cells resulting from antigen presentation migrate to the tissues most affected by GVHD, mainly the gastrointestinal tract, liver, skin, and lymphohematopoietic organs. These cells release inflammatory cytokines, which further augment GVHD.[2,20,23-26,52,58] Identifying early cytokine signatures after HCT can allow the clinician to anticipate the severity of the GVHD[58] and treat accordingly, and modulating or blocking these same cytokines also can help to prevent GVHD[25] (Figure 3). Most current therapeutic approaches to reduce GVHD rely on systemic immunosuppression (Figure 3). More refined approaches directed toward the modulation of T cells are being investigated[20] and will be required to prevent current pan-immunosuppressive strategies, which increase the rate of infection and relapse[15,16] (Figure 4). Preclinical evidence has shown that transplantation-related toxicities and lethal GVHD are reduced markedly after nonmyeloablative HLA-mismatched HCT[59,83] (Figures 3 and 4). Many of these studies have been performed in both large and small animals.

Animal Models of GVHD

Excellent reviews have been written describing mouse models for GVHD and GVL.[71] Murine studies have been critical for determining immunologic and molecular mechanisms of disease and

1. Myeloablative regimen: chemotherapy or irradiation given to a recipient that kills all host hematopoietic and bone-marrow–derived cells (including stem cells) and requires a bone-marrow transplant to rescue the recipient lymphohematopoietic system.

2. Nonmyeloablative (or reduced intensity) regimen: when the chemotherapy–irradiation approach does not kill all host bone-marrow–derived cells. In this scenario, the recipient's lymphohematopoietic cells recover, and hematopoietic cell transplantation is not absolutely required.

3. Graft-versus-host disease (GVHD): a life-threatening condition in which the transplanted donor cells attack the recipient's tissues and organs (liver, gastrointestinal tract, lymphohematopoietic organs, lung and skin).

4. Graft-versus-leukemia (GVL): a response driven by the transplanted donor cells in which the host leukemia (or tumor) cells are identified and killed. Often the GVL response is complicated by the development of graft-versus-host disease when donor immune cells recognize antigens shared by the tumor and host tissues.

5. Mixed hematopoietic chimerism: a term describing the degree of donor-derived immune cells within the peripheral blood (or lymphohematopoietic organs) of the recipient. A full chimera is a recipient in which 100% of the hematopoietic cells are of donor origin. A mixed chimera is a recipient in which both donor and host lymphohematopioetic cells coexist. The chimerism can be high (for example, >50% donor-origin cells) or low (<20% donor-origin cells) and often is a subjective assessment based on the design of the transplant. Microchimerism is a degree of chimerism that requires PCR analysis to identify donor-derived cells. Microchimerism usually occurs when chimerism is below the detection limits of flow cytometry (that is, <1%).

6. Haploidentical transplant: a transplant in which the donor and recipient share a set of alleles. This situation occurs most commonly with the mother–son combination, who are exact 'half' matches of each other.

Figure 1. Important terms.

therapy.[42] However, preclinical observations in murine models using HCT often cannot be accurately extrapolated to man and are difficult to replicate in large animals, specifically in regard to transplantation-associated complications[3,5,88,91] (Figure 5).

For example, in vivo host and donor T-cell depletion can be readily and completely achieved in mice by using monoclonal antibodies against T cells[20] (Figure 3). Unfortunately, clinical translation of biologic responses as observed in mice cannot be achieved in humans (or other larger mammals, such as NHP and swine[10,40,92], Figure 5). These differences support the usefulness of large animals that more closely resemble the human scenario than do experiments in mouse models.[11,12,31,43]

Mice provide an excellent model to study biologic mechanisms, but large animals can better accommodate clinically relevant approaches and applications. The use of large preclinical animal models is therefore a logical next step in translating discoveries from rodent models. Among large animal species, swine have been used extensively,[46] in part because of the availability of an extensive panel of reagents.[34-36,61-66,74-80,84,100] Although NHP HCT models have the obvious advantage of similarity to humans, financial cost, breeding difficulties, small litter sizes, long gestation periods (close to a year), and dangers such as bites and potential exposure of serious zoonotic diseases such as B virus make primate HCT models problematic. As another model, dogs have been extensively used in BMT studies.[47] However, in addition to their smaller size compared with humans, dogs are not an optimal model for the study of GVHD because of their furred skin.

Compared with these other models, pigs are more economical, have shorter gestations (approximately 4 mo) and larger litters sizes (8 to 12 piglets), are easily handled and safe to work with, and engender less societal and ethical sensitivity with their use in biomedical research because they are already extensively used in the food industry. Their similarity to humans in terms of both organ size and physiology make miniature swine an excellent large animal model. The Major histocompatibility (MHC)-defined miniature swine permits transplantation scenarios that resemble human clinical situations, and with the availability of inbred lines, genetic studies similar to those achieved in mice can be performed

in swine. The availability of harvested spontaneous tumors within the most inbred lines[9,19] and their similarity to human neoplasias[19] make MGH pigs an ideal model to investigate clinically relevant HCT approaches.[19] Herein we describe the advantages of pigs as a clinically relevant large-animal model for the study of GVHD.

GVHD in Swine Undergoing HCT

GVHD in swine develops in a similar manner as that observed in humans after allogeneic HCT. Surpassing a minimal threshold of T cells is crucial for the development of GVHD, especially during myeloablative regimens[60,68] (Figure 4). Novel nonmyeloablative protocols that we developed suggest that the infusion of large numbers of alloreactive T cells is not sufficient to cause GVHD.[10]

In experimental minor-antigen–mismatched HCT studies in pigs, animals that received 900 cGy of total-body irradiation (TBI) and more than 7.5×10^8 nucleated BM cells/kg developed GVHD and survived long-term (Figure 4). When fewer than 7.5×10^8 BM cells/kg were delivered, pigs succumbed to infection or hemorrhage 30 to 45 d after transplantation[60,68] (Figure 4). Therefore, blood support and antibiotic, antiviral, and antifungal treatment is recommended (Figure 6).

In this context, nonlethal skin GVHD usually occurred. In pilot studies, skin GVHD was successfully controlled by using 1 to 3 doses (10 mg/kg each) of methyl prednisolone after a myeloablative regimen and minor-antigen–mismatched HCT. Recipients of T-cell–depleted donor BM did not survive long-term, as they succumbed to BM failure and infections.[60,68] Similar findings have been obtained in humans, in whom T-cell depletion of the allogeneic graft, although it significantly decreases the incidence of GVHD, has been associated with an increased incidence of relapse and graft loss[15].

In addition, allogeneic HCT have been performed across MHC barriers.[60,68,81,82] In a parent-into-F1 model, swine irradiated with 900 cGy TBI and given 2 to 9×10^8 BM cells/kg developed acute GVHD.[82] Recipients of nonT-cell–depleted BM grafts developed skin GVHD with a clinical course and pathology that resembled human skin GVHD.[81] In particular, the pig rashes occurred on the
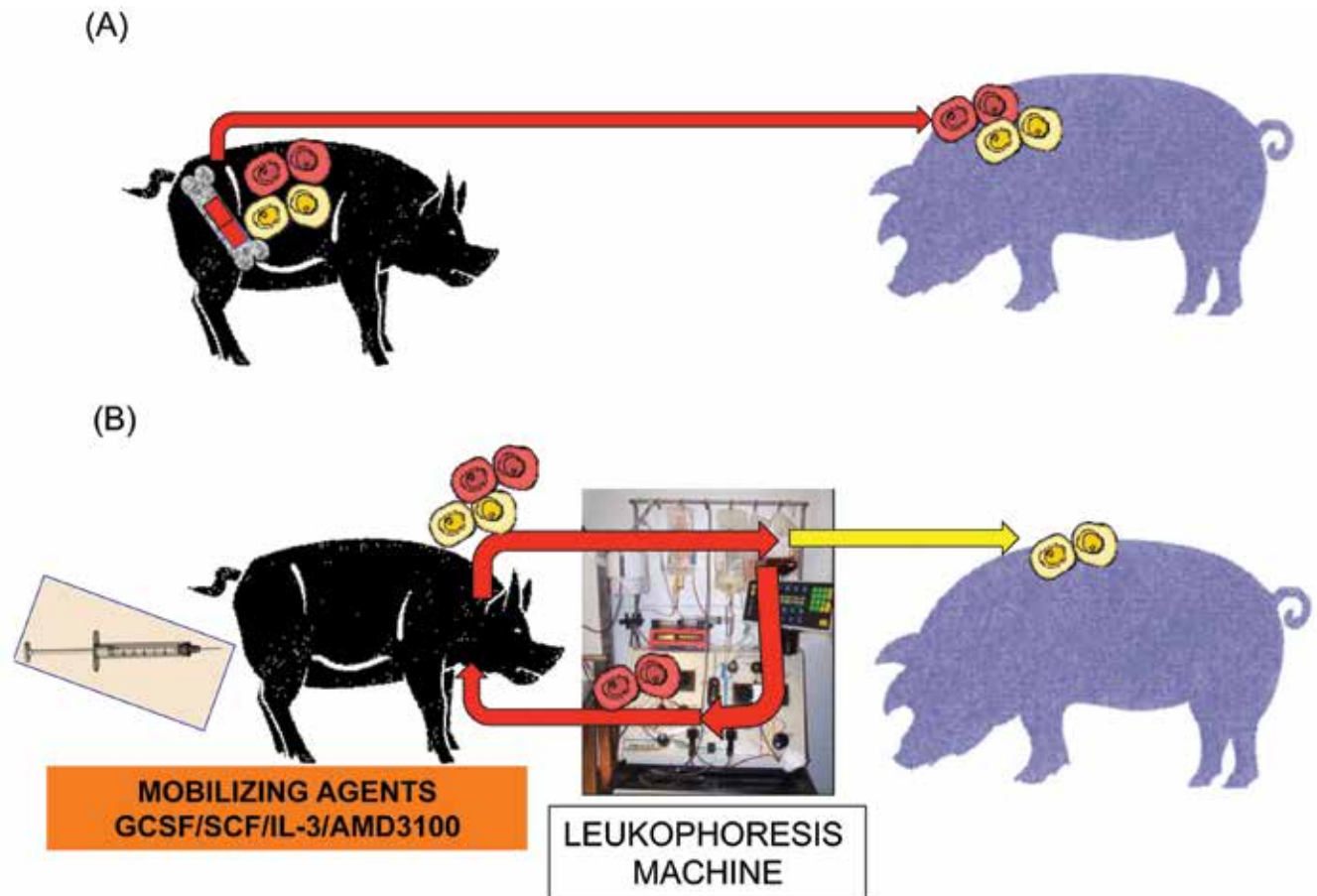
Figure 2. Two methods of harvesting hematopoietic stem cells for transplantation. (A) In bone marrow transplantation, hematopoietic stem cells are directly harvested from the iliac crests (or vertebrae, in the case of a nonsurviving donor). (B) Cytokine-mobilized hematopoietic cell transplantation (CM-HCT) is the most common approach to HCT clinically. Here, pigs (or humans) are injected with cytokines that target the release of the hematopoietic stem cells into the peripheral circulation. The most common agent used clinically in humans is GCSF. Additional cytokines used commonly in pigs have included porcine stem cell factor (SCF), porcine IL3, and AMD3100. The donor is catheterized, and a leukophoresis machine harvests all leukocytes and returns plasma, RBC, and platelets to the recipient. Compared with BMT, the T-cell content is usually larger with CM-HCT, potentially increasing the risk of GVHD. In addition, the total dose of CD34+ cells that can be delivered can be higher, because multiple phoresis can be performed. Once the IV catheters are in place, the donor does not need to be anesthetized for this procedure.

neck, back, and abdomen and often the lesions became confluent and ulcerative. GVHD usually presented 7 to 10 d after HCT. Some pigs had spontaneous resolution of their skin lesions, only to have a second episode of skin GVHD. If additional T cells were given (in the form of peripheral blood infusions), skin GVHD worsened in severity, and gastrointestinal GVHD developed with dysentery-like symptoms (as occurs in humans). Liver enzyme elevations were present also. Contrary to these findings, mice do not typically develop GVHD, even across full MHC barriers,[71] and require high doses of purified T cells (or bulk splenocytes) to induce GVHD. These differences between mice and pigs (and humans) support the case that, for preclinical translation, pigs represent a valuable extension of preliminary (mechanistic) murine studies and may be more suitable for assessing the clinical applicability of these novel approaches.

In MHC-mismatched transplants, when the donor BM was T-cell–depleted and after myeloablative conditioning of the host, pigs still developed GVHD, but it was milder in nature.[68,81,82] In a very few cases, recipients of T-cell–depleted haploidentical BMT and 900 cGy TBI never developed GVHD, and the pigs survived

long-term. The principal histopathologic difference between pig and human skin GVHD is that pigs have a denser infiltrate of neutrophils in addition to the classic lymphocytic infiltrate than humans.[82] The relevance is unknown and might reflect that pigs have contaminated skin surfaces or have evolved to have high neutrophil counts in the epithelium to prevent bacterial infections. Some pig studies demonstrated that, when peripheral blood T cells were added to T-cell–depleted BM, severe GVHD occurred, as expected. The difference in the severity and nature of GVHD suggested, however, that mature peripheral blood T cells are more aggressive than are BM-derived T cells in myeloablative regimens. Clinically speaking, T-cell contamination of a BM aspirate is mostly from the peripheral blood, and minimizing the number of alloreactive T cells has been a major subject of clinical research. Similar to the situation in pigs, ex vivo T-cell depletion of a human graft is complicated by higher rates of engraftment failure.[15]

For the past 15 y, cytokine-mobilized HCT have been performed in MGH pigs. The results of these studies have closely paralleled human clinical findings. The use of allogeneic HCT—

| Treatment type	Target
Cellular	
Regulatory T cells (Tregs)	T cells, B cells, antigen-presenting cells, monocytes
Mesenchymal stromal/stem cells (MSCs)	Tcells, B cells, antigen-presenting cells, PMNs, NK cells
Nonpharmacologic	
Reduced-intensity conditioning	Decreased chemotherapy- and irradiation-induced organ damage
Extracorporeal photophoresis	T cells
Pharmacologic	
Glucocorticoids	Lymphocytes, monocytes
Calcineurin inhibitors (for example, tacrolimus, cyclosporine)	T cells
mTOR inhibitors (for example, rapamycin)	T cells (and enhance regulatory T cells)
Mycophenolate mofetil	T cells
Pentostatin	T cells and NK cells
Antithymocyte globulin	T cells
AntiCD3, antiCD2, antiCD4, and antiCD8 monoclonal antibodies	T cells
AntiCD20 monoclonal antibodies	B cells
AntiTNFα monoclonal antibody (Infliximab)	T cells
TNF receptor inhibitor (Etanercept)	T cells

Figure 3. GVHD treatment approaches.
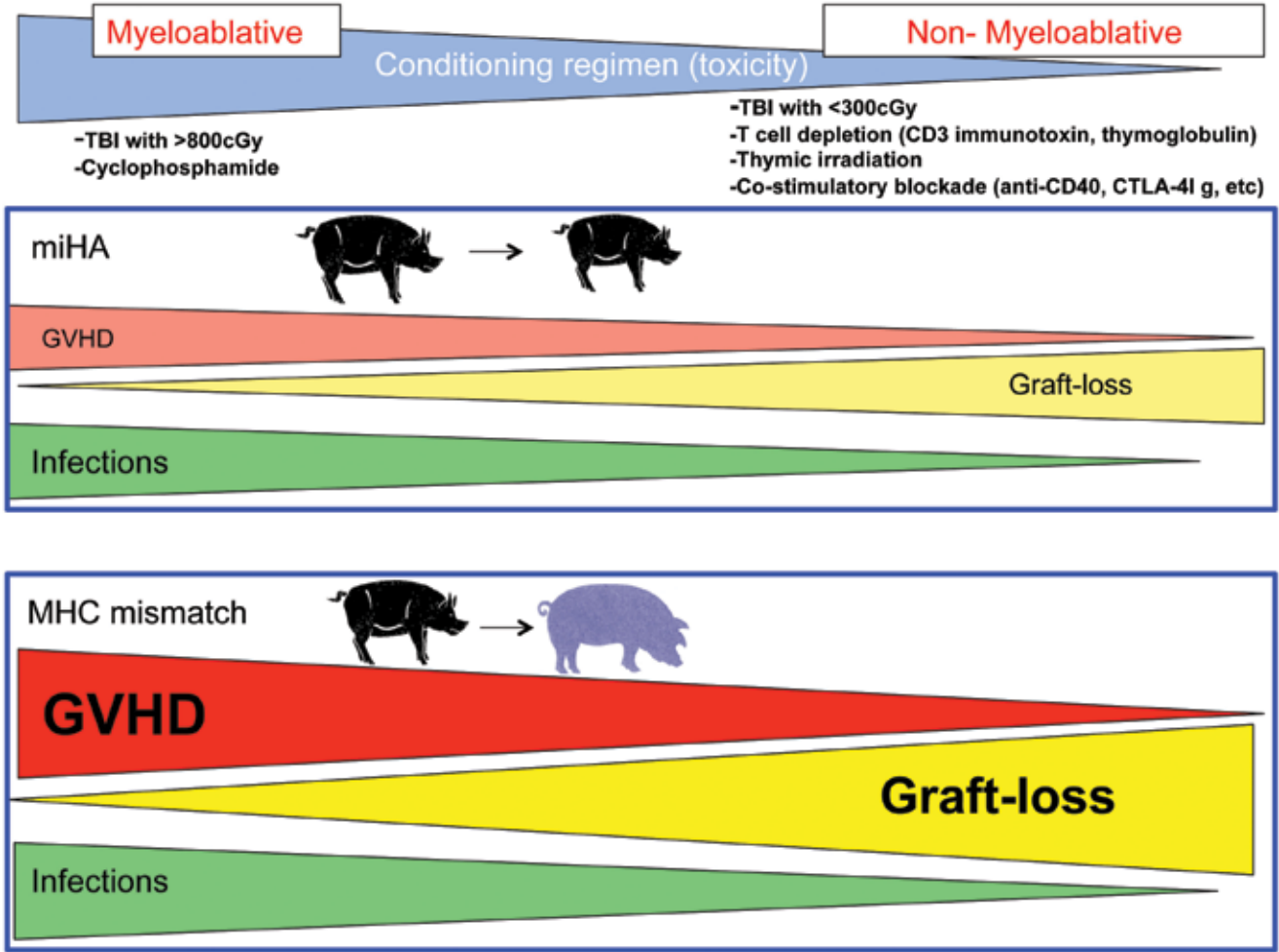
Figure 4. Likelihood of infection, GVHD, and graft loss according to the toxicity of the preparatory regimen and the degree of MHC mismatch. Pigs that undergo myeloablative HCT are more likely to develop infections and thus require more intensive antifungal, antiviral, antibiotic prophyllaxis. These animals also have a higher risk of GVHD because of the chemotherapy and irradiation treatments and the inflammation associated with the regimen. Graft loss typically is minimal under these conditions. In contrast, when the preparatory regimen is mild, the likelihood of infection is reduced greatly, but the infused donor graft may be at higher potential of being lost (that is, rejected by the host). The incidence of GVHD under these conditions is decreased. GVHD tends to be more severe across MHC barriers but less severe in minor-antigen–mismatched (miHA) transplantation. The most potent antitumor effects are provided by MHC-mismatched transplantation.

	Mice	Pigs	Rationale
Mechanistic studies	+++	—	Availability of mouse strains allows for 'clean,' highly reproducible, and controlled immunologic studies.
Skin GVHD	—	+++	Histopathologic lesions and anatomy are more similar between pigs and humans.
Clinical infectious disease complications	—	+	Closed and SPF status in mice prevent many common complications observed in humans. Swine are outbred and carry viruses (such as herpesviruses) which, when reactivated, resemble the pathologies observed in humans undergoing transplantation.
T cell depletion	+++	—	Swine (as humans) do not achieve the levels of T-cell depletion achieved in mice
GVHD induction with BM alone	—	+	Swine resemble humans, in that additional T cells are not required to induce GVHD.
Cell dose for clinical application	—	+	Swine are infused with cell doses that mimic the clinical scenario.
Cytokine-mobilized HCT	—	+	Mobilization approaches are practical in pigs and more clinically applicable. Blood volume and vascular access is comparable between humans and pigs.
Preparatory regimens for transplantation	—	+	Responses to chemotherapeutics and irradiation in swine mirror those in humans with greater fidelity than do rodents.
GVL studies	+++	—	Graft-versus-tumor (or leukemia) studies cannot be performed in swine. A reliable model has not yet been established.

Figure 5. Relative benefits of small (mouse) and large (swine) animal models for the study of BMT and GVHD

like HCT across MHC barriers—has been limited by GVHD when myeloablative regimens are used. Novel protocols to minimize GVHD, aimed to induce a state of immunologic tolerance, have been developed by using MGH miniature swine.[10,27-30,38,39,45] To that end, many preparatory regimens have been nonmyeloablative (also referred to as 'reduced-intensity conditioning' regimens). These nonmyeloablative, haploidentical HCT resulted in a reduced incidence and intensity of GVHD.[27,39] We previously published[10] that reduced-intensity conditioning regimens consisting of 100 cGy TBI, CD3 immunotoxin treatment, and 45 d of cyclosporine followed by the delivery of 15×10^9 mobilized haploidentical PBMC per kilogram were associated with no or only limited (and nonlife-threatening) GVHD. We are investigating the mechanisms behind this novel approach by which alloreactive T cells are delivered (and controlled). Although donor T cells are involved in the pathogenesis of GVHD, the presence of large numbers of alloreactive T cells alone is often not sufficient to cause GVHD. As of 2015, 63 pigs have undergone a reduced-intensity conditioning regimen consisting of low-dose TBI (100 cGy), T-cell depletion with an antiCD3 immunotoxin, and cyclosporine A monotherapy for 45 d. Of these 63 animals, only 8 developed GVHD after cyclosporine A was discontinued, and only 2 of these 8 developed severe, acute GVHD. One of the pigs that developed severe acute GVHD received an HCT inoculum from a donor that had an adverse response (disseminated intravascular coagulation secondary to mobilization regimen),[50] which might have been, at least in part, responsible for GVHD. It is probable that the disruption or dysregulation of immune regulatory mechanisms through either harsh conditioning or adverse reactions were involved also.

The use of donor leukocyte infusions as a therapy for cancer after HCT has been studied in pigs (Duran-Struuck and colleagues, manuscript in preparation). In the clinic, donor leukocyte infusions usually are used as a tool to drive strong antitumor responses. These therapies are also useful in inducing hematopoietic conversion from mixed hematopoietic chimerism to full-donor chimerism. Unfortunately, full conversion often is complicated by

GVHD. Therefore, the development of a reliable protocol using donor leukocyte infusion in mixed chimeras to provide strong GVH responses and eliminate tumor without GVHD (as has been demonstrated in murine studies[73]) is needed. To this end, miniature swine have been used to translate these novel approaches.

Preliminary experiments in MGH miniature swine demonstrated that donor leukocyte infusions in stable, long-term, mixed chimeras were ineffective at increasing the levels of donor chimerism. Of the 30 donor leukocyte infusions delivered experimentally to pigs, 9 demonstrated a response measured either by conversion, bone marrow failure, or GVHD (Duran-Struuck and colleagues manuscript in preparation). These pigs received doses of 50×10^6 T cells/kg or greater (that is, high relative to doses used clinically but and at the low end of the range in murine experiments). Similar to what is observed in humans that convert after donor leukocyte infusion, GVHD developed. Thus a better understanding of the immunobiology of GVHD in a large-animal model of allogeneic HCT for clinical applications is needed. We now discuss the strengths of pigs as a model for GVHD.

Swine, humans, and target organs of GVHD

The clinical and histopathologic presentation of GVHD in swine closely mimics what is observed in humans.[82] We have developed the 'Seattle' GVHD scoring system for swine in collaboration with the MGH clinical HCT program (Figure 7). To assess the suitability of swine as an accurate animal model of GVHD, we compared the target organs affected by GVHD, namely the liver, skin, secondary lymphoid organs, and gastrointestinal tract.

The skin is the most commonly affected target tissue of GVHD in both humans and swine.[17,22,25] Numerous studies describe the similarities and differences in skin histology between swine and humans. Both have well-defined dermal papillae and rete ridges. Although pig skin is thicker and less vascular than that of humans, the overall characteristics of the cutaneous blood supply are similar.[46] The size, distribution, and orientation of blood vessels in the dermis of pigs
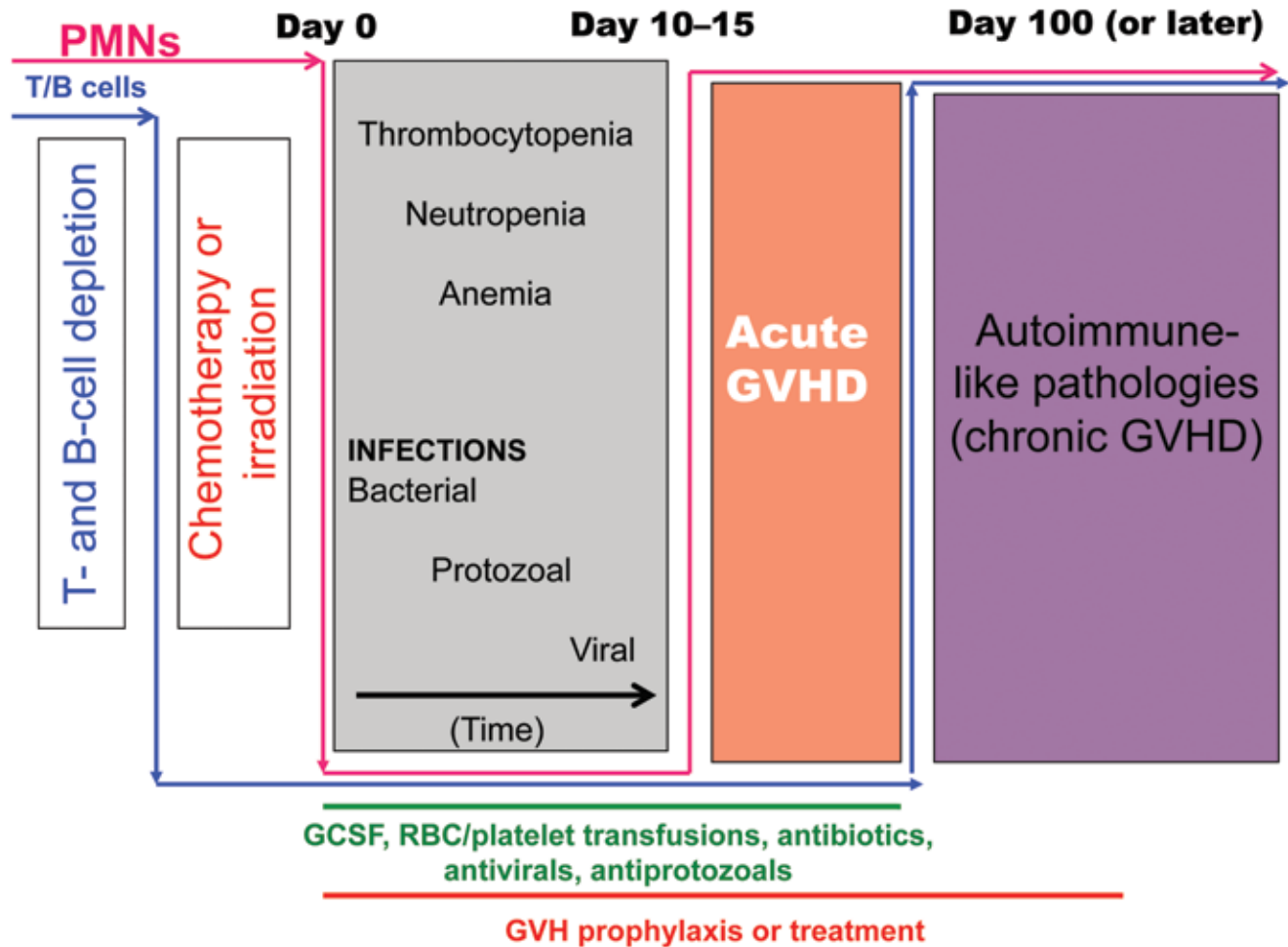
Figure 6. BMT with marked myelosuppression. Because of their involvement in acute and chronic GVHD, controlling T and B cells is important in this scenario, and new approaches aimed at eliminating memory B and T cells are key. T and B cells are necessary for viral control and clearance, and their depletion places the recipient at an increased risk of virus-related infections. In addition, chemotherapeutics and irradiation treatments destroy the myeloid lineages, particularly polymorphonuclear cells and monocytes, which are important for defense against bacterial and protozoal infections. Therefore recipients require antibiotics, antivirals, and antifungals. Furthermore, recipients typically are at risk of bleeding during the first 2 to 3 wk after transplantation. Thrombocytopenia (platelets, PLT) and anemias are usual, and blood support may be required, depending on the severity of the regimen. Blood support becomes less necessary during the third or fourth week after transplantation, as hematopoietic lineages recover. Control of acute GVHD is important during the first 100 d after transplantation; often calcineurin inhibitors (such as cyclosporin) or mTOR inhibitors (such as rapamycin) are administered to this end. Chronic GVHD occurs later after transplantation and resembles antibody-mediated autoimmune conditions. In these cases, B-cell–depleting agents, such as rituximab, are used. Of note, the CD20 monoclonal antibody does not work in pigs but does in NHP.

are similar to those found in human skin. However, the subepidermal plexus, which supplies adnexal structures, is less developed in pigs, although the adnexal structures found in swine and humans are similar.[49,51] Physiologically, swine and human skin have many similarities. These include the epidermal turnover time, type of keratinous proteins found within the skin, and lipid composition of the stratum corneum.[49,51,53] Based on these similarities, pigs are an excellent model for studying skin GVHD, in contrast to other species such as mice, dogs, and NHP, all of which are furred animals. Clinically, swine GVHD exhibits a similar course to that in humans. The initial presentation is in the form of an erythematous rash that can progress to severe and generalized hyperkeratosis and ulceration. Because of these many similarities, we clinically score the GVHD in our pigs according to the same parameters as described in humans. For practical reasons, we include a drawing of a pig on which we note the level of skin involvement (Figures 8 and 9). We also photograph

and mark the edges of the erythema for assessment of resolution or progression of skin GVHD. Grossly, early GVHD can be confused with many skin conditions, including a drug (hypersensitivity) rash or a herpetic skin condition, but advanced GVHD is unequivocal (Figure 8). When untreated (or uncontrolled), skin GVHD can be lethal in pigs, just like in humans. The histopathology of skin GVHD in pigs is similar to what has been documented in humans.[94] Lymphocytic infiltration along the dermal–epidermal junction is observed, especially at the rete tips (Figure 9). Satellitosis, which is the surrounding of lymphocytes around keratinocytes undergoing apoptosis, is also seen. Hyperkeratosis and ulceration is present in grade IV GVHD (Figures 8 and 9).

GVHD affects the gastrointestinal tract as well[25] (Figure 10) and can involve either the upper (stomach, duodenum, jejunum) or lower (rectum, colon) regions. Histologically, the stomachs of both humans and pigs have a glandular epithelium, although pigs also

TBRC SWINE CLINICAL GVHD ASSESSMENT R Duran 2010

Date Of Assessment:
Transplant Day:

STAGE

	0	1	2	3	4	
Skin	☐	☐	☐	☐	☐	% body rash:
Upper GI	☐	☐				
Lower GI	☐	☐	☐	☐	☐	Volume stool: Color stool
Liver	☐	☐	☐	☐	☐	

SKIN (draw below)	UPPER GI	LOWER GI
0 = No rash	0 = No nausea and vomiting	0 = no diarrhea
1 = Maculopapular rash on < 25% of BSA	1 = Persistent nausea, vomiting or anorexia	1 = soft stools
2 = Maculopapular rash on 25% to 50%		2 = diarrhea
3= Rash > 50%, generalized erythroderma		3 = bloody diarrhea
4 = Stage 3 plus bullae and desquamation		4= b diarrh+hunched

LIVER (max total=4)

Bilirubin	ALKP	ALT	AST
0 = < 0.8 mg/dl	0=<294	0= <43	0=<65
0.25 = 0.8 – 1.5 mg/dl	0.25=294-500	0.25=43-90	0.25=65-130
0.5 = 1.5 – 3 mg/dl	0.5=500-1500	0.50.0=90-180	0.50=130-500
0.75 = 3 – 9 mg/dl	0.75=1500-3000	0.75=180-400	0.75=500-1500
1= > 9.0mg/dl	1.0=>3000	1.0=>400	1=>1500
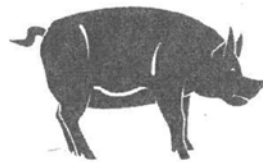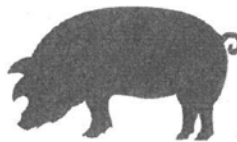
RIGHT   LEFT

Overall Grading of Acute GVHD

	₀☐ Grade 0	A☐ Grade 1	B☐ Grade 2	C☐ Grade 3	D☐ Grade 4
SKIN	0	1	2	3	4
LOWER GI	0	0	1-2	3	4
UPPER GI	0	0	1		
LIVER	0	0	1-2	3	4

Note: Subscripts relate to grading at MGH clinic

Figure 7. Seattle GVHD scoring system adapted for use with swine.

have a muscular and mucoid glandular structure called the torus pyloricus, which is located near the pyloric sphincter. This structure is unique in pigs and is involved in the functional closure of the pylorus. The pig small intestine, as in humans, functions as the major site for absorption and consequently has a large surface area with finger-shaped villi. However, the pig gastrointestinal
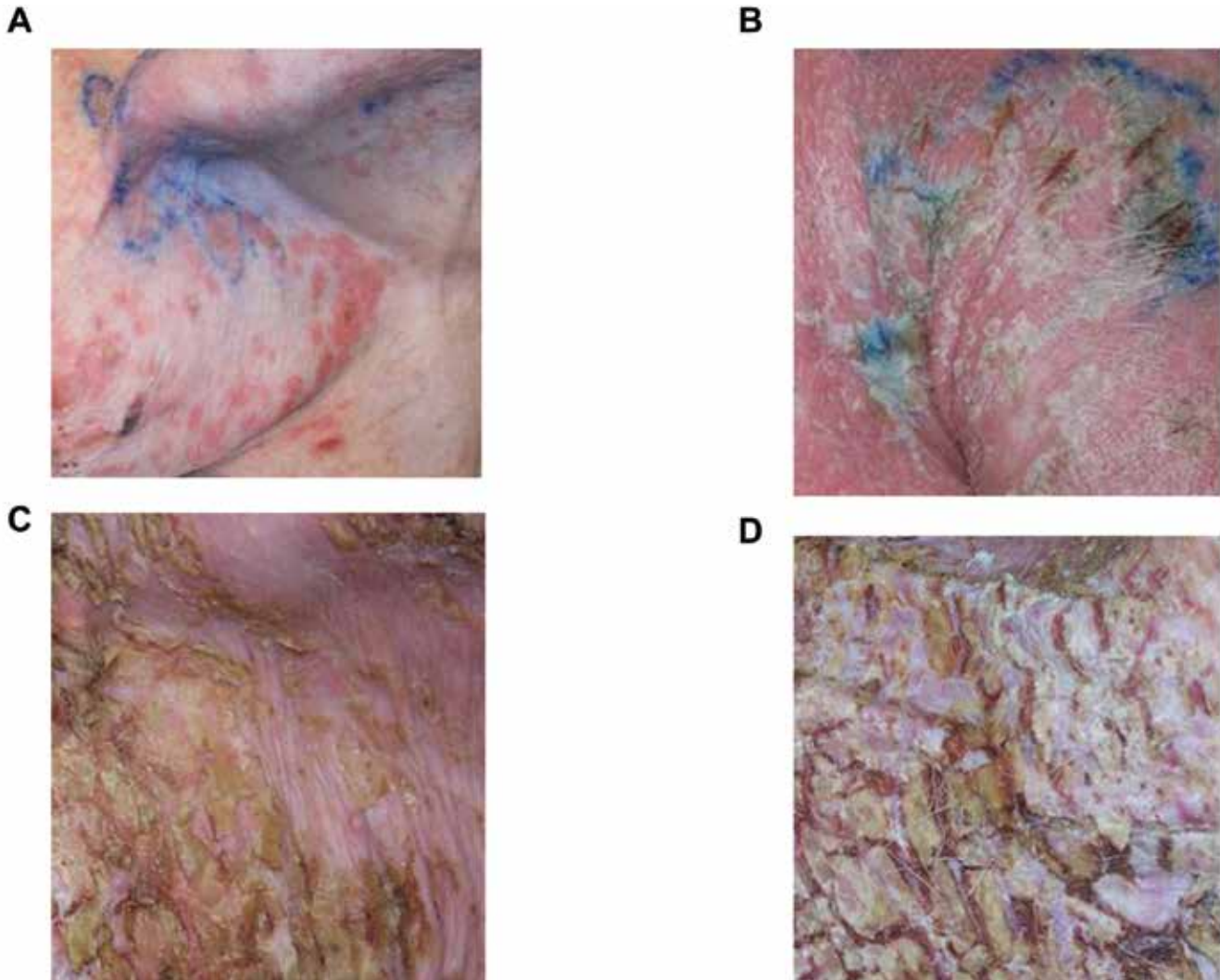
Figure 8. Clinical skin GVHD. (A) Maculopapular rash characteristic of GVHD observed in and near high-motion areas on day 56 after HCT. The left inguinal area is shown; the head of the pig is to the left. (B) Generalized erythema and desquamation of the left lateral leg on day 62 after HCT. Erythema now extends beyond the inguinal high-motion area to the lateral aspect of the leg. (C) Moderate hyperkeratosis and mild ulceration of the medial left inguinal area on day 70 after HCT. (D) Severe hyperkeratosis cracking of skin and severe ulcerations observed in the left inguinal region on day 76 after HCT. The blue pigment is ink used to identify the borders of the erythematous regions, to assess the kinetics of the skin GVHD.

tract is very long, extending as much as 15 times the length of the body.[46] The anatomy of the colon is significantly different in pigs. It has a centrifugal and centripetal loop, thus coiling within itself and occupying a large part of the abdominal cavity. Physiologically, pigs and humans are both omnivores, and they share similar characteristics in regard to digestion and intestinal transport.[19] Indeed, this likeness may also help to explain their similarity in liver metabolism (discussed in following section). Given these considerations, we grade gastrointestinal GVHD in pigs similarly to the scoring that is performed in humans (Figure 7).

From a practical standpoint, the availability of metabolic cages for large animals also permits us to easily quantify the volume of diarrhea produced when gastrointestinal GVHD develops. The level of dehydration can be assessed by physical exams by using eyelid, axillary, or inguinal skin tenting and through bloodwork. Because all of our pigs have central venous catheters, supportive care with crystalloids, colloids, or any type of blood product for

the management of the GVHD is relatively simple. Furthermore, we monitor serum and hematologic parameters without sedating the pigs (this process is more difficult and associated with a higher risk in awake NHP models).

The liver is frequently affected by GVHD (Figures 10 B and 11). Pigs have been readily used as an animal model for hepatic studies.[1,93] One study claims that the metabolic functions of human liver may be more similar to those of swine than they are to those of NHP species.[18] Grossly, the size and shape of the liver between human and pigs is comparable.[46,89] Histopathologically, the pig liver is very similar to that of humans, with the major difference being the connective tissue septae that demarcate the hepatic lobes and lobules. Human livers normally lack connective tissue in these areas.

An important physiologic function of the liver in both humans and swine is the synthesis of albumin, the most abundant protein in plasma. Human and porcine albumin show 65% similarity at
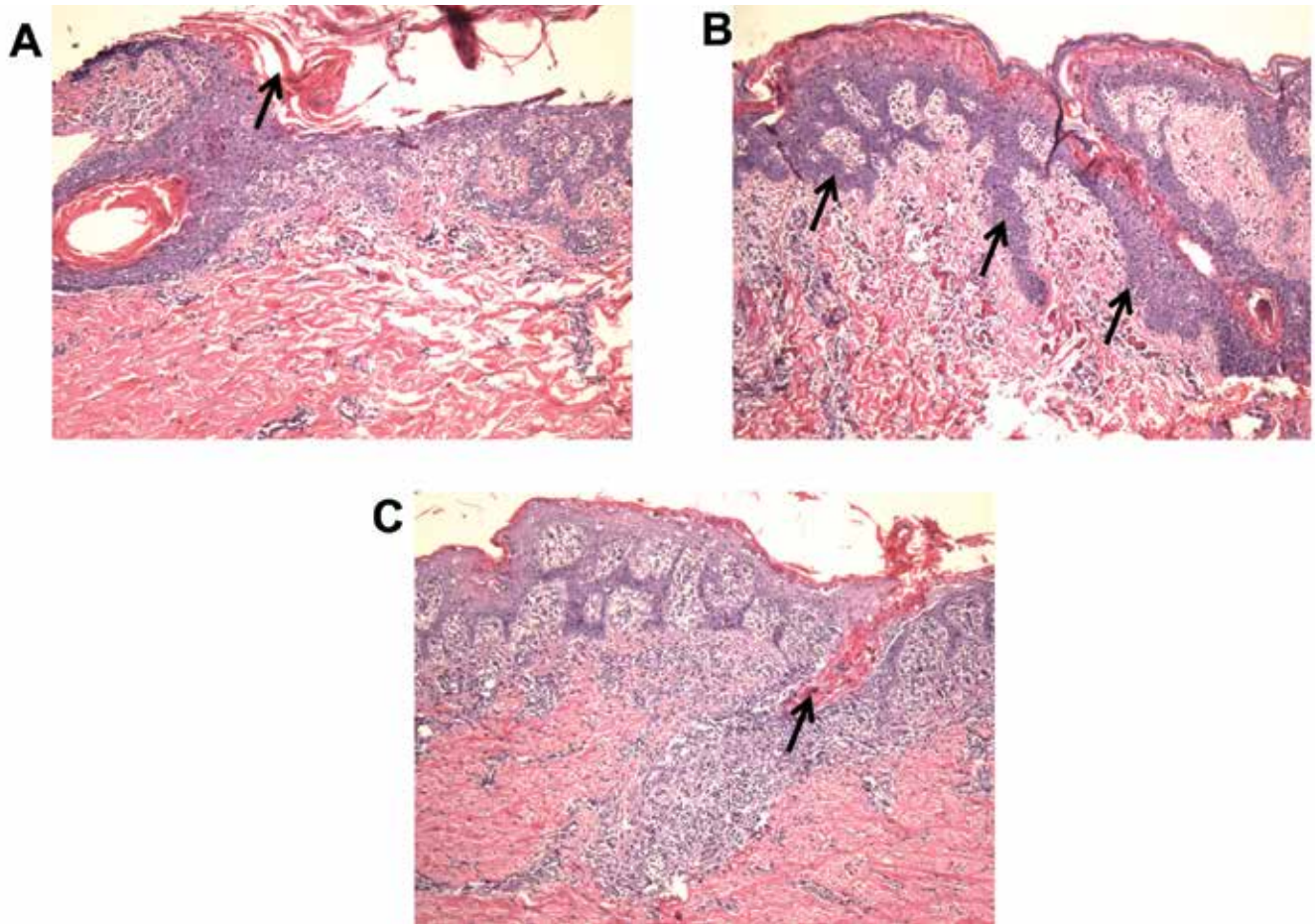
Figure 9. Histopathologic lesions of GVHD. (A) Skin from pig 18432 on day 56 after haploidentical HCT. There is mild mononuclear inflammatory cell infiltrate in the superficial dermis. Mixed inflammatory cells, including neurophils, infiltrate the epidermis. The crust on the surface of the skin is composed of exudates and keratin (solid arrow). Hematoxylin and eosin stain; magnification, 10×. (B) Skin from from same animal on day 62 after HCT. There is mild to moderate inflammatory cell infiltration in the superficial dermis and epidermis (arrows). The superficial epidermis is necrotic. Capillaries are congested. Hematoxylin and eosin stain; magnification, 10×. (C) Day 76 after HCT. There is extensive mononuclear cell infiltrate in the superficial dermis and around a hair follicle (arrow). The epidermis is acantholytic and hyperkeratotic. Hematoxylin and eosin stain; magnification, 10×.

the amino-acid level, although the serum albumin concentration is lower in pigs than in primates.[33,67] Furthermore, one study[44] found no significant difference in the composition of hepatic bile, including viscosity, between humans and pigs. These similarities allow assessment and comparison of bile stasis in human patients and swine with liver GVHD.

Serum bilirubin concentrations can provide a measure of functional capacity of the liver and are closely followed and used for GVHD scoring clinically.[25] Pigs with GVHD, similar to what is observed in humans, have elevated serum levels of bilirubin and various liver enzymes (Figure 11 B and C). Histopathologic diagnosis (Figure 10 B) of GVHD documents the presence of a lymphoid infiltrate and correlates with increases in chimerism (Figure 11 A) and bilirubin (Figure 11 C).

The cytochrome P450 system has a similar activity between pigs and humans.[87] Blood glucose is closely monitored in pigs with GVHD. In contrast to humans, who are able to maintain blood glucose through extrahepatic gluconeogenesis (mainly by the kidney, gut and muscle),[48] anhepatic pigs are unable to maintain blood glucose concentrations, with blood lactate levels rising.[48] The mechanisms of cholesterol transport also differ between pigs and humans. The lipoprotein complexes used to transport cholesterol in the blood (LDL, HDL, and VLDL) differ between the 2 species by 40% at the protein level.[33] In addition, pigs have a lower binding capacity of LDL, with a greater number of apoB receptors in the liver.[41]

The different effects of liver GVHD on cholesterol and its related metabolites between pigs and humans are unclear, but we continue to monitor these serum parameters as a component of hepatic functional assessment in our studies and to obtain additional information regarding the overall health status of our miniature swine. Both complement and coagulation factors are crucial in host defense and clotting mechanisms. Studies from several groups suggest that differences in complement formation and coagulation factors exist between pigs and humans. However, 2 studies in which baboons received transgenic pig livers documented that clotting parameters normalized after the xenotransplanted liver was placed.[21,69]

The use of Banna inbred minipig clotting factors demonstrated that swine serum successfully activates the human intrinsic and extrinsic clotting pathways. Interestingly, the activities of some factors (II, V, VII, XII) were significantly higher in pigs than hu-
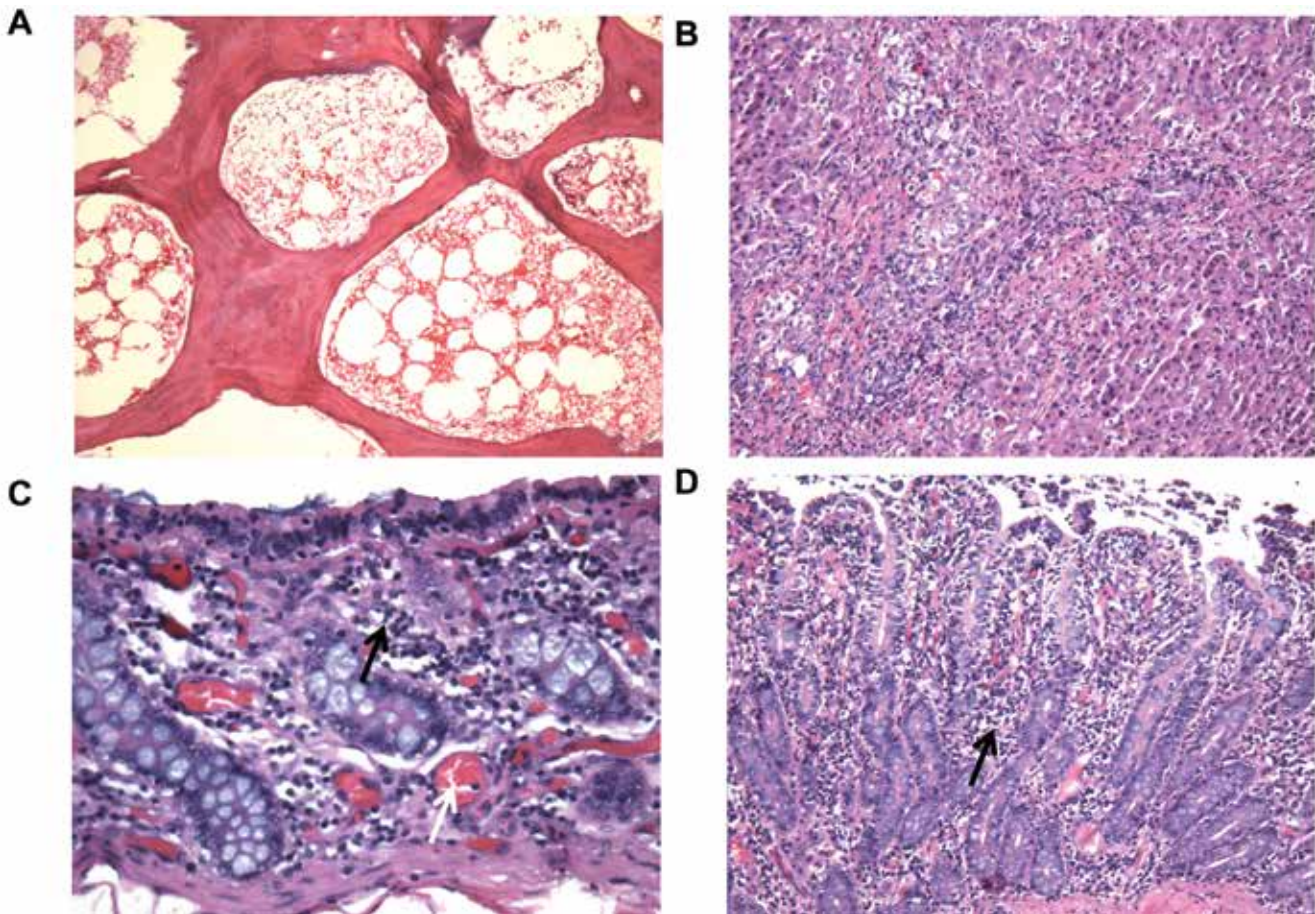
Figure 10. Swine GVHD histopathology of BM, liver, and small and large intestine. (A) BM from a pig that underwent BM failure secondary to lymphohematopoietic GVHD after a halpoidentical HCT and donor lymphocyte infusion. There are very few cells with nuclei in the marrow, which is composed primarily of adipocytes and RBC. Normal BM shows numerous nucleated cells. Hematoxylin and eosin stain; magnification, 10×. (B) Liver from a pig that developed GVHD 30 d after haploidentical HCT. A moderately severe mononuclear inflammatory cell infiltration is present in the portal areas and bridges from portal area to portal area. The adjacent parenchyma is infiltrated slightly, as well. Hematoxylin and eosin stain; magnification, 20×. (C) Colon from the same animal as in panel A. The lamina propria is infiltrated by moderate numbers of lymphocytes. The capillaries are congested. Hematoxylin and eosin stain; magnification, 40×. (D) Small intestine from the pig shown in panel A. The lamina propria is densely infiltrated by lymphocytes. Hematoxylin and eosin stain; magnification, 20×.

mans.[98,99] A study involving the use of pig liver xenoperfusion to treat acute liver failure in humans detected increased levels of vitamin-K-independent clotting factors (V and XII) and decreased levels of vitamin-K-dependent factors VII and X in pigs.[4] Pigs in our allogeneic HCT studies are assessed for clotting deficiencies and coagulation factors once liver GVHD is suspected.

Hepatic veno-occlusive disease is a serious complication of HCT in humans,[37] but to date, we have not diagnosed this condition in any of our pigs. Therefore, similar to humans, in whom the incidence of veno-occlusive disease is minimal in reduced-intensity conditioning regimens, pigs undergoing similar preparatory regimens do not develop veno-occlusive disease.

The pig liver produces complement proteins as well,[70] and complement-regulatory proteins are thought to be relatively species-specific. However, in some experiments, human complement-regulatory protein was able to inhibit porcine complement,[72] thus suggesting that the proteins are relatively compatible. Therefore, in theory, the effect of liver GVHD on complement formation may be similar between humans and pigs.

Given the similarities in liver anatomy and physiology between humans and pigs, our liver GVHD scoring system for pigs includes evaluations of total bilirubin, ALT, AST, and ALP (Figure 7), all of which increase when GVHD develops (Figure 11 B and C). In our GVHD scoring system, we have assigned equal importance to the ALP, ALT, AST, and total bilirubin levels. In addition, liver GVHD in pigs can be followed longitudinally, and biopsies can be obtained by means of laparotomy or transcutaneous ultrasound-guided methods as clinically indicated (Figure 10 B).

The lymphoid organs—mainly the lymph nodes, spleen, thymus, and BM—are targets of GVHD as well as the sites where allogeneic immune responses are primed.[25] The most important gross and histopathologic differences between swine and humans are in the characteristics of the lymph nodes and thymus. The lymph nodes of swine display a unique histologic characteristic: the typical cortex and medulla are reversed, with the germinal centers located in the interior of the gland.[46] Afferent lymph moves through the node from the central cortex to the outer paracortex, which is equivalent to the medulla. The cells then travel through the endothelial venules and back into the blood. Despite
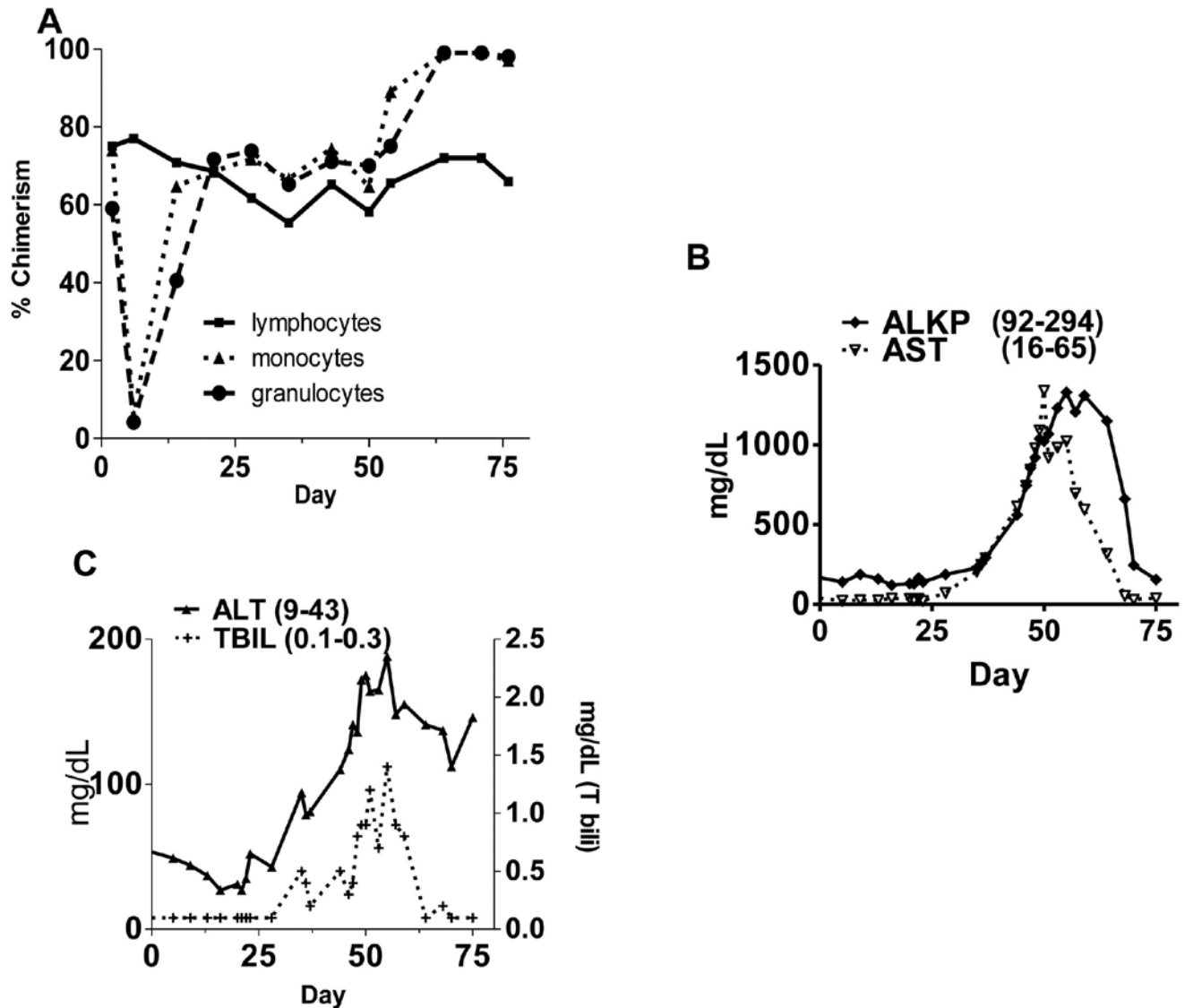
Figure 11. Chimerism, weight loss, and liver parameters associated with GVHD. (A) This pig developed acute GVHD soon after transplantation. The animal developed liver GVHD on day 30, during the cyclosporine taper. Weight loss observed starting on day 55, with GVHD affecting the liver and skin. Donor-derived cell populations are given in % (*y* axis, amount of donor chimerism; *x* axis, time [d] after HCT). (B) AST, ALP, and total bilirubin and (C) ALT.

these anatomic differences, lymph node follicles and cellular and humoral immune responses are elicited in swine in a similar fashion to what occurs in humans and other species.[7,8,85,86] Although lymphocytes are similar between swine and humans in many respects, some studies have revealed interesting differences.[32] For example, peripheral lymphocytes in swine include CD4+CD8+ αβ T cells and high numbers of γδ T cells. Many of the γδ T cells are CD2+. CD2 is also found on some CD8 α– cells (including NK cells) and conventional γδ and αβ T cells. Conversely, the porcine T-cell receptor β repertoire is highly conserved and nearly identical to that in humans.[86] The spleen in swine functions primarily as a clearance and RBC storage organ and has poorly developed or nonexistent sinuses. In addition, lymphocyte turnover in the porcine spleen is estimated to be 30 times higher than that in the circulation.[56,57] Anatomically, the spleen of pigs is long and thin and wraps around the greater curvature of the stomach, unlike the spleen of humans, which is smaller and more kidney-like in appearance.

The pig thymus is histologically similar to those of other species.[86] The thymus has a thin connective tissue capsule, which penetrates and divides it into different lobules. The outer cortex is darkly stained with packed small lymphocytes that are separate from the medulla. The medulla is paler and is continuous between lobes but may appear isolated and surrounded by the cortex, depending on histology. Grossly, the pig has a very large thymus, part of which extends into the thoracic cavity. As a result, thymic biopsies are very easy to perform and are minimally invasive, because there is no need to enter the thoracic cavity. The spleen and thymus are targets of the GVHD response. Due to destruction, thymus tissue is difficult to find in pigs that develop GVHD. BM failure as a result of GVHD is not uncommon (Figure 10 A). The thymus in swine involutes with age, as occurs in humans,[54,55,95-97] thus T cell

output diminishes as it atrophies. Although comparative studies detailing the incidence of GVHD between aged and young swine have not been performed, we anticipate that, given the available literature, the incidence of GVHD in pigs might vary according to age, similar to what has been observed in humans and mice.[6] Splenic, BM, thymic, and lymph node biopsies can be performed easily in swine, and the ability to assess peripheral lymphoid organs (in addition to blood) longitudinally after HCT makes pigs an invaluable animal model for the study of GVHD.

Future Directions

In summary, because of their physiologic and anatomic similarities to humans, pigs likely will provide increasing opportunities to explore novel preventive and therapeutic approaches to GVHD after MHC-matched and -mismatched HCT. Unlike rodent models, miniature swine provide the ability to perform extended longitudinal studies on individual animals, because multiple tissue biopsies can be taken without requiring euthanasia.

Future preclinical studies in the MGH miniature swine can exploit the genetics (MHC characterization and inbred lines) of the model with their similarities to human clinical outcomes. Adoptive transfer studies with MGH swine, one of our most inbred lines (coefficient of inbreeding, >97%), can be a powerful tool for the understanding of responses to cellular targets (such as regulatory T cells) with minimal genetic variation between animals. Novel swine studies can be performed to evaluate clinically controversial issues such as the role of mesenchymal stem (stromal) cells in HCT (Figure 3). In addition, in-depth investigation of the role of $\gamma\delta$ and $\alpha\beta$ T cells in GVHD can be performed easily in swine.

Given the similarities between human and pig skin, novel GVHD therapies such as extracorporeal photopheresis and topical treatments can be readily assessed in this species. The availability of swine tumor cell lines and our current efforts to develop a swine tumor model will, for the first time, permit for the investigation of novel antitumor cellular and pharmacologic treatments in a large-animal model.[9,19] The similarity in the responses to preparatory regimens between pigs and humans reinforces the choice of this large-animal model for studies of HCT.

Novel preparatory regimens, which can be translated to the clinic for the development of tolerance, have already been extensively investigated in our laboratory. It is important to understand whether these approaches enhance or control the development of GVHD. Given the advantages mentioned previously, MGH miniature swine can provide the translational bridge between mouse and humans and will likely be instrumental in the testing of new antiGVHD therapeutic approaches.

Materials and Methods

Transplant donors and recipients were selected from the herd of partially inbred, MHC-defined MGH miniature swine. A breeding pair, one pig from the Andes and a second one from the Rockies, were selected for the creation of the herd. MGH miniature swine are a closed SPF herd (free of pseudorabies, porcine reproductive and respiratory syndrome, transmissible gastroenteritis viruses and brucellosis) of the species *Sus scrofa domestica* that is defined at the MHC loci. Approximately 50 miniature swine of varying ages and weights are housed in our large animal facility. The MGH is an AAALAC-accredited facility. Animals undergoing HCT are housed in conventional steel cages with HEPA filters.

Donor animals shown in the figures ranged from 6 mo to 1 y old; recipients were 8 to 12 wk of age. Donors and recipients were chosen to differ by single haplotypes at both the class I and class II loci. All donors were chosen to be positive for pig allelic antigen (PAA), a nonhistocompatibility cell-surface antigen that is present on all differentiated hematopoietic cells in animals that express this allele. All recipients were chosen to be PAA-negative to detect chimerism by flow cytometry after HCT. All transplantations were approved by the Massachusetts General Hospital IACUC.

Preparatory regimen. Miniature swine SLA[ad] recipient animals (age, 8 to 12 wk; weight, 8 to 12 kg) were pretreated with low-dose (100 cGy) TBI on day −2, partial T-cell depletion by using a CD3 immunotoxin delivered intravenously prior to HCT, and a 45-d course of oral cyclosporine A (referred to as the 'ITC nonmyeloablative conditioning regimen') followed by megadose haploidentical HCT. Beginning on day −4 until day −1, recipients received 50 µg/kg of a recombinant CD3 immunotoxin twice daily, 8 h apart. Pigs were premedicated with 2 mg/kg diphenhydramine, and the immunotoxin was administered by intravenous push followed by flushing with PBS. Cyclosporine was administered through a gastrostomy tube twice daily, beginning on day −1 and concluding on day 45. Target (therapeutic) levels were 400 to 800 ng/mL from day −1 to day 30, followed by a steady taper to day 45, at which point cyclosporine levels were lower than 200 ng/mL and thus considered subtherapeutic.

Donor cytokine mobilization and HCT. Miniature swine SLA[ac] donor animals (age, 6 to 12 mo; weight, 40 to 60 kg) underwent hematopoietic stem-cell mobilization with IL3 and porcine stem cell factor at a dose of 100 µg/kg for the first 30 kg of body weight and 50 µg/kg for the remaining body weight. Porcine-specific cytokines were developed by Biotransplant (Charlestown, MA) and supplied either through Biotransplant or the Dana Farber Recombinant Protein Expression and Purification Core facility at Massachusetts General Hospital. Cytokines were administered beginning on day −5 and concluding on day 2 or until the pig was deemed clinically unfit to continue. Animals (were sedated with 1 to 2 mg/kg tiletamine–zolazepam prior to subcutaneous administration of cytokines (including porcine IL3 and stem cell factor). PBMC were harvested by using 8 to 10 h of leukapheresis beginning on day 0. Thereafter, donor animals were leukapheresed while unanesthetized on days 1 and 2 until the target cell dose of 15×10^9 cells/kg was achieved or pigs were clinically unfit to continue. After each leukapheresis, cells were infused into conditioned recipient pigs at a rate of 20 mL/kg hourly. Donor mobilizations and subsequent recipient HCT outcomes were retrospectively compared with those previously published.[6]

References

1. **[Anonymous].** 1984. The piglet as a model for perinatal fatty acid metabolism in man. Nutr Rev **42:**257–258.

2. **Abhyankar S, Gilliland DG, Ferrara JL.** 1993. Interleukin 1 is a critical effector molecule during cytokine dysregulation in graft-versus-host disease to minor histocompatibility antigens. Transplantation **56:**1518–1522.

3. **Adelman CA, Petrini JH, Attwooll CL.** 2006. Modeling disease in the mouse: lessons from DNA damage response and cell cycle control genes. J Cell Biochem **97:**459–473.

4. **Adham M, Ducerf C, Vernet M, Rigal D, de la Roche E, Bizollon T, Taibi A, Pouyet M, Baulieux J.** 1997. Changes in serum proteins during isolated pig liver xenoperfusion. Transplant Proc **29:**3015.

5. **Bortin MM.** 1970. A compendium of reported human bone marrow transplants. Transplantation **9:**571–587.

6. **Bryson JS, Jennings CD, Caywood BE, Dix AR, Lowery DM, Kaplan AM.** 1997. Enhanced graft-versus-host disease in older recipient mice following allogeneic bone marrow transplantation. Bone Marrow Transplant **19:**721–728.

7. **Butler JE, Sinkora M, Wertz N, Holtmeier W, Lemke CD.** 2006. Development of the neonatal B and T cell repertoire in swine: implications for comparative and veterinary immunology. Vet Res **37:**417–441.

8. **Butler JE, Sun J, Wertz N, Sinkora M.** 2006. Antibody repertoire development in swine. Dev Comp Immunol **30:**199–221.

9. **Cho PS, Lo DP, Wikiel KJ, Rowland HC, Coburn RC, McMorrow IM, Goodrich JG, Arn JS, Billiter RA, Houser SL, Shimizu A, Yang YG, Sachs DH, Huang CA.** 2007. Establishment of transplantable porcine tumor cell lines derived from MHC inbred miniature swine. Blood **110:**3996–4004.

10. **Cina RA, Wikiel KJ, Lee PW, Cameron AM, Hettiarachy S, Rowland H, Goodrich J, Colby C, Spitzer TR, Neville DM Jr, Huang CA.** 2006. Stable multilineage chimerism without graft versus host disease following nonmyeloablative haploidentical hematopoietic cell transplantation. Transplantation **81:**1677–1685.

11. **Cohen JL, Trenado A, Vasey D, Klatzmann D, Salomon BL.** 2002. CD4$^+$CD25$^+$ immunoregulatory T cells: new therapeutics for graft-versus-host disease. J Exp Med **196:**401–406.

12. **Colson YL, Christopher K, Glickman J, Taylor KN, Wright R, Perkins DL.** 2004. Absence of clinical GVHD and the in vivo induction of regulatory T cells after transplantation of facilitating cells. Blood **104:**3829–3835.

13. **Cooke KR, Hill GR, Crawford JM, Bungard D, Brinson YS, Delmonte J Jr, Ferrara JL.** 1998. Tumor necrosis factor α production to lipopolysaccharide stimulation by donor cells predicts the severity of experimental acute graft-versus-host disease. J Clin Invest **102:**1882–1891.

14. **Cooke KR, Olkiewicz K, Erickson N, Ferrara JL.** 2002. The role of endotoxin and the innate immune response in the pathophysiology of acute graft-versus-host disease. J Endotoxin Res **8:**441–448.

15. **Copelan EA.** 2006. Hematopoietic stem-cell transplantation. N Engl J Med **354:**1813–1826.

16. **Copelan EA, Kapoor N, Berliner M, Tutschka PJ.** 1989. Bone marrow transplantation without total-body irradiation in patients aged 40 and older. Transplantation **48:**65–67.

17. **Deane M, Singer C, Lawler M, McElwaine S, Gomez K, Prentice HG.** 1998. Acute skin GVHD following syngeneic BMT for CLL. Bone Marrow Transplant **22:**1207–1209.

18. **Drougas JG, Barnard SE, Wright JK, Sika M, Lopez RR, Stokes KA, Williams PE, Pinson CW.** 1996. A model for the extended studies of hepatic hemodynamics and metabolism in swine. Lab Anim Sci **46:**648–655.

19. **Duran-Struuck R, Cho PS, Teague AG, Fishman B, Fishman AS, Hanekamp JS, Moran SG, Wikiel KJ, Ferguson KK, Lo DP, Duggan M, Arn JS, Billiter B, Horner B, Houser S, Yeap BY, Westmoreland SV, Spitzer TR, McMorrow IM, Sachs DH, Bronson RT, Huang CA.** 2010. Myelogenous leukemia in adult inbred MHC-defined miniature swine: a model for human myeloid leukemias. Vet Immunol Immunopathol **135:**243–256.

20. **Duran-Struuck R, Reddy P.** 2008. Biological advances in acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Transplantation **85:**303–308.

21. **Ekser B, Echeverri GJ, Hassett AC, Yazer MH, Long C, Meyer M, Ezzelarab M, Lin CC, Hara H, van der Windt DJ, Dons EM, Phelps C, Ayares D, Cooper DK, Gridelli B.** 2010. Hepatic function after genetically engineered pig liver transplantation in baboons. Transplantation **90:**483–493.

22. **Esteban JM, Somlo G.** 1995. Skin biopsy in allogeneic and autologous bone marrow transplant patients: a histologic and immunohistochemical study and review of the literature. Mod Pathol **8:**59–64.

23. **Ferrara JL.** 1998. The cytokine modulation of acute graft-versus-host disease. Bone Marrow Transplant **21 Suppl 3:**S13–S15.

24. **Ferrara JL, Abhyankar S, Gilliland DG.** 1993. Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1. Transplant Proc **25:**1216–1217.

25. **Ferrara JL, Levine JE, Reddy P, Holler E.** 2009. Graft-versus-host disease. Lancet **373:**1550–1561.

26. **Ferrara JL, Levy R, Chao NJ.** 1999. Pathophysiologic mechanisms of acute graft-vs-host disease. Biol Blood Marrow Transplant **5:**347–356.

27. **Fuchimoto Y, Huang CA, Yamada K, Shimizu A, Kitamura H, Colvin RB, Ferrara V, Murphy MC, Sykes M, White-Scharf M, Neville DM Jr, Sachs DH.** 2000. Mixed chimerism and tolerance without whole body irradiation in a large animal model. J Clin Invest **105:**1779–1789.

28. **Fuchimoto Y, Yamada K, Shimizu A, Yasumoto A, Sawada T, Huang CA, Sachs DH.** 1999. Relationship between chimerism and tolerance in a kidney transplantation model. J Immunol **162:**5704–5711.

29. **Gleit ZL, Cameron AM, Fuchimoto Y, Melendy E, Monajati L, Coburn RC, Sachs DH, Huang CA.** 2002. Persistent chimerism despite antidonor MHC in vitro responses in miniature swine following allogeneic hematopoietic cell transplantation. Transplantation **74:**1260–1266.

30. **Gleit ZL, Fuchimoto Y, Yamada K, Melendy E, Scheier-Dolberg R, Monajati L, Coburn RC, Neville DM Jr, Sachs DH, Huang CA.** 2002. Variable relationship between chimerism and tolerance following hematopoietic cell transplantation without myelosuppressive conditioning in miniature swine. Transplantation **74:**1535–1544.

31. **Gonzalez M, Quezada SA, Blazar BR, Panoskaltsis-Mortari A, Rudensky AY, Noelle RJ.** 2002. The balance between donor T-cell anergy and suppression versus lethal graft-versus-host disease is determined by host conditioning. J Immunol **169:**5581–5589.

32. **Haley PJ.** 2003. Species differences in the structure and function of the immune system. Toxicology **188:**49–71.

33. **Hammer C.** 1998. Potential barriers to xenogeneic organ transplantation. Schweiz Med Wochenschr **128:**931–934.

34. **Haverson K, Bailey M, Stokes CR, Simon A, LeFlufy L, Banfield G, Chen Z, Hollemweguer E, Ledbetter JA.** 2001. Monoclonal antibodies raised to human cells—specificity for pig leukocytes. Vet Immunol Immunopathol **80:**175–186.

35. **Haverson K, Saalmuller A, Alvarez B, Alonso F, Bailey M, Bianchi AT, Boersma WJ, Chen Z, Davis WC, Dominguez J, Engelhardt H, Ezquerra A, Grosmaire LS, Hamilton MJ, Hollemweguer E, Huang CA, Khanna KV, Kuebart G, Lackovic G, Ledbetter JA, Lee R, Llanes D, Lunney JK, McCullough KC, Molitor T, Nielsen J, Niewold TA, Pescovitz MD, de la Lastra JM, Rehakova Z, Salmon H, Schnitzlein WM, Seebach J, Simon A, Sinkora J, Sinkora M, Stokes CR, Summerfield A, Sver L, Thacker E, Valpotic I, Yang H, Zuckermann FA, Zwart R.** 2001. Overview of the Third International Workshop on Swine Leukocyte Differentiation Antigens. Vet Immunol Immunopathol **80:**5–23.

36. **Haverson K, Saalmuller A, Chen Z, Huang CA, Simon A, Seebach J, Boersma WJ, Zwart R, Niewold TA, Thacker E, Llanes D, de la Lastra JM, Engelhardt H, Ezquerra A, Alonso F, Dominguez J, Ledbetter JA, Grosmaire L, Lee R, Nielsen J, Salmon H, Valpotic I, Sver L, Lackovic G, Summerfield A, Khanna KV.** 2001. Summary of the first round analyses of the Third International Workshop on

Swine Leukocyte Differentiation Antigens. Vet Immunol Immuno-pathol **80:**25–34.

37. **Ho VT, Revta C, Richardson PG.** 2007. Hepatic veno-occlusive disease after hematopoietic stem cell transplantation: update on defibrotide and other current investigational therapies. Bone Marrow Transplant **41:**229–237.

38. **Horner BM, Cina RA, Wikiel KJ, Lima B, Ghazi A, Lo DP, Yamada K, Sachs DH, Huang CA.** 2006. Predictors of organ allograft toler-ance following hematopoietic cell transplantation. Am J Transplant **6:**2894–2902.

39. **Huang CA, Fuchimoto Y, Scheier-Dolberg R, Murphy MC, Neville DM Jr, Sachs DH.** 2000. Stable mixed chimerism and tolerance using a nonmyeloablative preparative regimen in a large-animal model. J Clin Invest **105:**173–181.

40. **Huang CA, Yamada K, Murphy MC, Shimizu A, Colvin RB, Neville DM Jr, Sachs DH.** 1999. *In vivo* T cell depletion in miniature swine using the swine CD3 immunotoxin pCD–CRM9. Transplantation **68:**855–860.

41. **Huff MW, Telford DE, Edmonds BW, McDonald CG, Evans AJ.** 1993. Lipoprotein lipases, lipoprotein density gradient profile, and LDL receptor activity in miniature pigs fed fish oil and corn oil. Biochim Biophys Acta **1210:**113–122.

42. **Hunter KW, Williams RW.** 2002. Complexities of cancer research: mouse genetic models. ILAR J **43:**80–88.

43. **Johnson BD, Konkol MC, Truitt RL.** 2002. CD25+ immunoregulatory T cells of donor origin suppress alloreactivity after BMT. Biol Marrow Transplant **8:**525–535.

44. **Kobayashi T, Taniguchi S, Ye Y, Niekrasz M, Nour B, Cooper DK.** 1998. Comparison of bile chemistry between humans, baboons, and pigs: implications for clinical and experimental liver xenotransplan-tation. Lab Anim Sci **48:**197–200.

45. **Kunisaki SM, Haller GW, Fuchimoto Y, Huang CA, Sachs DH.** 2001. Peripheral regulation of graft-versus-host alloreactivity in mixed chimeric miniature swine. Transplantation **72:**523–526.

46. **Laber KE, Whary MT, Bingel SA, Goodrich JA, Smith AC, Swindle MM.** 2002. Biology and diseases of swine. In: **Fox JG, Anderson LC, Loew FM, Quimby FW,** editors. Laboratory animal medicine, 2nd ed. London (United Kingdom): Academic Press.

47. **Ladiges WC, Storb R, Thomas ED.** 1990. Canine models of bone marrow transplantation. Lab Anim Sci **40:**11–15.

48. **Lauritsen TL, Grunnet N, Rasmussen A, Secher NH, Quistorff B.** 2002. The effect of hepatectomy on glucose homeostasis in pig and in man. J Hepatol **36:**99–104.

49. **Lavker RM, Dong G, Zheng PS, Murphy GF.** 1991. Hairless mi-cropig skin. A novel model for studies of cutaneous biology. Am J Pathol **138:**687–697.

50. **Matar AJ, Crepeau RL, Pathiraja V, Robson S, Fishman JA, Spitzer TR, Sachs DH, Huang CA, Duran-Struuck R.** 2012. Effects of mo-bilization regimens in donors on outcomes of hematopoietic cell transplantation in miniature swine. Comp Med **62:**487–494.

51. **Miller SJ, Burke EM, Rader MD, Coulombe PA, Lavker RM.** 1998. Re-epithelialization of porcine skin by the sweat apparatus. J Invest Dermatol **110:**13–19.

52. **Molldrem JJ, Schlomchik WD.** 2005. Graft-versus-leukemia effects, p 155–194. In: **Ferrara JLM, Cooke KR, Deeg HJ,** editors. Graft-versus-host disease. New York (NY): Marcel Dekker.

53. **Nicolaides N, Fu HC, Rice GR.** 1968. The skin surface lipids of man compared with those of 18 species of animals. J Invest Dermatol **51:**83–89.

54. **Nobori S, Samelson-Jones E, Shimizu A, Hisashi Y, Yamamoto S, Kamano C, Teranishi K, Vagefi PA, Nuhn M, Okumi M, Wong B, Houser S, Sachs DH, Yamada K.** 2006. Long-term acceptance of fully allogeneic cardiac grafts by cotransplantation of vascularized thymus in miniature swine. Transplantation **81:**26–35.

55. **Nobori S, Shimizu A, Okumi M, Samelson-Jones E, Griesemer A, Hirakata A, Sachs DH, Yamada K.** 2006. Thymic rejuvenation and the induction of tolerance by adult thymic grafts. Proc Natl Acad Sci USA **103:**19081–19086.

56. **Pabst R, Kaatz M, Westermann J.** 1983. In situ labelling of bone marrow lymphocytes with fluorescein isothiocyanate for lymphocyte migration studies in pigs. Scand J Haematol **31:**267–274.

57. **Pabst R, Westermann J.** 1991. The role of the spleen in lymphocyte migration. Scanning Microsc **5:**1075–1079.

58. **Paczesny S, Krijanovski OI, Braun TM, Choi SW, Clouthier SG, Kuick R, Misek DE, Cooke KR, Kitko CL, Weyand A, Bickley D, Jones D, Whitfield J, Reddy P, Levine JE, Hanash SM, Ferrara JL.** 2009. A biomarker panel for acute graft-versus-host disease. Blood **113:**273–278.

59. **Pelot MR, Pearson DA, Swenson K, Zhao G, Sachs J, Yang YG, Sykes M.** 1999. Lymphohematopoietic graft-vs-host reactions can be induced without graft-vs-host disease in murine mixed chime-ras established with a cyclophosphamide-based nonmyeloablative conditioning regimen. Biol Blood Marrow Transplant **5:**133–143.

60. **Pennington LR, Sakamoto K, Popitz-Bergez FA, Pescovitz MD, McDonough MA, MacVittie TJ, Gress RE, Sachs DH.** 1988. Bone marrow transplantation in miniature swine. I. Development of the model. Transplantation **45:**21–26.

61. **Pescovitz MD, Book BK, Aasted B, Dominguez J, Bullido R, Trebichavsky I, Novikov B, Valpotic I, Nielsen J, Arn S, Sachs DH, Lunney JK, Boyd PC, Walker J, Lee R, Petrinec N, Saalmuller A.** 1998. Analyses of monoclonal antibodies reacting with porcine wCD6: results from the Second International Swine CD Workshop. Vet Immunol Immunopathol **60:**285–289.

62. **Pescovitz MD, Book BK, Aasted B, Dominguez J, Bullido R, Trebi-chavsky I, Novikov B, Valpotic I, Tomaskovic M, Nielsen J, Arn S, Sachs DH, Lunney JK, Boyd PC, Walker J, Lee R, Saalmuller A.** 1998. Analyses of monoclonal antibodies reacting with porcine CD5: results from the Second International Swine CD Workshop. Vet Immunol Immunopathol **60:**269–273.

63. **Pescovitz MD, Book BK, Aasted B, Dominguez J, Ezquerra A, Trebichavsky I, Novikov B, Valpotic I, Nielsen J, Arn S, Sachs DH, Lunney JK, Boyd PC, Walker J, Lee R, Lackovic G, Kirkham P, Park-house RM, Saalmuller A.** 1998. Analyses of monoclonal antibodies reacting with porcine CD3: results from the Second International Swine CD Workshop. Vet Immunol Immunopathol **60:**261–268.

64. **Pescovitz MD, Book BK, Aasted B, Dominguez J, Ezquerra A, Trebichavsky I, Novikov B, Valpotic I, Sver L, Nielsen J, Arn S, Sachs DH, Lunney JK, Boyd PC, Walker J, Lee R, Davis W, Barbosa IR, Zuckermann F, Saalmuller A.** 1998. Summary of workshop findings for antibodies reacting with porcine T cells and activation antigens: results from the Second International Swine CD Workshop. Vet Immunol Immunopathol **60:**251–260.

65. **Pescovitz MD, Hsu SM, Katz SI, Lunney JK, Shimada S, Sachs DH.** 1990. Characterization of a porcine CD1-specific mAb that distinguishes CD4/CD8 double-positive thymic from peripheral T lymphocytes. Tissue Antigens **35:**151–156.

66. **Pescovitz MD, Lunney JK, Sachs DH.** 1984. Preparation and char-acterization of monoclonal antibodies reactive with porcine PBL. J Immunol **133:**368–375.

67. **Platt JL.** 2000. Physiologic barriers to xenotransplantation. Transplant Proc **32:**1547–1548.

68. **Popitz-Bergez FA, Sakamoto K, Pennington LR, Pescovitz MD, McDonough MA, MacVittie TJ, Gress RE, Sachs DH.** 1988. Bone marrow transplantation in miniature swine. II. Effect of selective genetic differences on marrow engraftment and recipient survival. Transplantation **45:**27–31.

69. **Ramirez P, Chavez R, Majado M, Munitiz V, Munoz A, Hernandez Q, Palenciano CG, Pino-Chavez G, Loba M, Minguela A, Yelamos J, Gago MR, Vizcaino AS, Asensi H, Cayuela MG, Segura B, Marin F, Rubio A, Fuente T, Robles R, Bueno FS, Sansano T, Acosta F, Rodriguez JM, Navarro F, Cabezuelo J, Cozzi E, White DJ, Calne RY, Parrilla P.** 2000. Life-supporting human complement regulator decay accelerating factor transgenic pig liver xenograft maintains the metabolic function and coagulation in the nonhuman primate for up to 8 days. Transplantation **70:**989–998.

70. **Ramirez P, Montoya MJ, Rios A, Garcia** Palencianco **C, Majado M, Chavez R, Munoz A, Fernandez OM, Sanchez A, Segura B, Sansano T, Acosta F, Robles R, Sanchez F, Fuente T, Cascales P, Gonzalez F, Ruiz D, Martinez L, Pons JA, Rodriguez JI, Yelamos J, Cowan P, d'Apice A, Parrilla P.** 2005. Prevention of hyperacute rejection in a model of orthotopic liver xenotransplantation from pig to baboon using polytransgenic pig livers (CD55, CD59, and H transferase). Transplant Proc **37:**4103–4106.

71. **Reddy P, Negrin R, Hill GR.** 2008. Mouse models of bone marrow transplantation. Biol Blood Marrow Transplant **14:**129–135.

72. **Rees MA, Butler AJ, Negus MC, Davies HF, Friend PJ.** 2004. Classical pathway complement destruction is not responsible for the loss of human erythrocytes during porcine liver perfusion. Transplantation **77:**1416–1423.

73. **Rubio MT, Kim YM, Sachs T, Mapara M, Zhao G, Sykes M.** 2003. Antitumor effect of donor marrow graft rejection induced by recipient leukocyte infusions in mixed chimeras prepared with nonmyeloablative conditioning: critical role for recipient-derived IFNγ. Blood **102:**2300–2307.

74. **Saalmuller A, Aasted B, Canals A, Dominguez J, Goldman T, Lunney JK, Maurer S, Pauly T, Pescovitz MD, Pospisil R, Salmon H, Trebichavsky I, Valpotic I, Vizcaino JS, Weiland E, Zuckermann F.** 1994. Analyses of monoclonal antibodies reactive with porcine CD6. Vet Immunol Immunopathol **43:**243–247.

75. **Saalmuller A, Aasted B, Canals A, Dominguez J, Goldman T, Lunney JK, Pauly T, Pescovitz MD, Pospisil R, Salmon H, Sinkora J, Summerfield A, Valpotic I, Vizcaino JS, Zuckermann F.** 1994. Analysis of mAb reactive with the porcine SWC1. Vet Immunol Immunopathol **43:**255–258.

76. **Saalmuller A, Aasted B, Canals A, Dominguez J, Goldman T, Lunney JK, Maurer S, Pescovitz MD, Pospisil R, Salmon H, Summerfield A, Tlaskalova H, Valpotic I, Vizcaino JS, Weiland E, Zuckermann F.** 1994. Analyses of monoclonal antibodies reactive with porcine CD5. Vet Immunol Immunopathol **43:**237–242.

77. **Saalmuller A, Aasted B, Canals A, Dominguez J, Goldman T, Lunney JK, Maurer S, Pescovitz MD, Pospisil R, Salmon H, Tlaskalova H, Valpotic I, Vizcaino JS, Weiland E, Zuckermann F.** 1994. Summary of workshop findings for porcine T-lymphocyte antigens. Vet Immunol Immunopathol **43:**219–228.

78. **Saalmuller A, Aasted B, Canals A, Dominguez J, Goldman T, Lunney JK, Pauly T, Pescovitz MD, Pospisil R, Salmon H, Sinkora J, Summerfield A, Valpotic I, Vizcaino JS, Zuckermann F.** 1994. Analysis of mAb reactive with the porcine SWC1. Vet Immunol Immunopathol **43:**255–258.

79. **Saalmuller A, Denham S, Haverson K, Davis B, Dominguez J, Pescovitz MD, Stokes CC, Zuckermann F, Lunney JK.** 1996. The Second International Swine CD Workshop. Vet Immunol Immunopathol **54:**155–158.

80. **Saalmuller A, Pauly T, Aasted B, Jensen KT, Sachs DH, Arn S, Davis WC, Park YH, McCullough K, Summerfield A, Murtaugh M, Pampusch MS, Burger KD, Laber J, Nielsen J, Pescovitz MD, Stokes C, Haverson K, Boyd P, Lunney JK.** 1998. Summary of the first round analyses of the Second International Swine CD Workshop. Vet Immunol Immunopathol **60:**237–249.

81. **Sakamoto K, Pennington LR, Popitz-Bergez FA, Pescovitz MD, Gress RE, McDonough MA, Shimada S, Katz SI, Sachs DH.** 1987. Swine GVHD model and the effect of T-cell depletion of marrow by monoclonal antibodies, p. 449–453. In: **Gale RP, Champlin R,** editors. Progress in bone marrow transplantation. New York (NY): Alan R Liss.

82. **Sakamoto K, Sachs DH, Shimada S, Popitz-Bergez FA, Pennington LR, Pescovitz MD, McDonough MA, MacVittie TJ, Katz SI, Gress RE.** 1988. Bone marrow transplantation in miniature swine.

III. Graft-versus-host disease and the effect of T-cell depletion of marrow. Transplantation **45:**869–875.

83. **Sharabi Y, Sachs DH.** 1989. Mixed chimerism and permanent specific transplantation tolerance induced by a nonlethal preparative regimen. J Exp Med **169:**493–502.

84. **Sinkora J, Rehakova Z, Haverson K, Sinkora M, Dominguez J, Huang CA.** 2001. Monoclonal antibodies putatively recognising activation and differentiation antigens. Vet Immunol Immunopathol **80:**143–164.

85. **Sinkora M, Butler JE.** 2009. The ontogeny of the porcine immune system. Dev Comp Immunol **33:**273–283.

86. **Sinkora M, Butler JE, Holtmeier W, Sinkorova J.** 2005. Lymphocyte development in fetal piglets: facts and surprises. Vet Immunol Immunopathol **108:**177–184.

87. **Skaanild MT, Friis C.** 1997. Characterization of the P450 system in Gottingen minipigs. Pharmacol Toxicol **80 Suppl 2:**28–33.

88. **Storb R.** 2003. Allogeneic hematopoietic stem cell transplantation—yesterday, today, and tomorrow. Exp Hematol **31:**1–10.

89. **Swindle MM, Makin A, Herron AJ, Clubb FJ Jr, Frazier KS.** 2011. Swine as models in biomedical research and toxicology testing. Vet Pathol **49:**344–356.

90. **Teshima T, Ferrara JL.** 2002. Understanding the alloresponse: new approaches to graft-versus-host disease prevention. Semin Hematol **39:**15–22.

91. **van Bekkum DW.** 1984. Conditioning regimens for marrow grafting. Semin Hematol **21:**81–90.

92. **Wang Z, Duran-Struuck R, Crepeau R, Matar A, Hanekamp I, Srinivasan S, Neville DM** Jr, **Sachs DH, Huang CA.** 2011. Development of a diphtheria-toxin-based antiporcine CD3 recombinant immunotoxin. Bioconjug Chem. **22:**2014–2020.

93. **Wolf P, Meyer C, Boudjema K, Kieny R, Cinqualbre J, Jaeck D, Andre E, Herrenschmidt N, Azimzadeh A.** 1997. The pig as a model in liver xenotransplantation. Vet Res **28:**217–222.

94. **Wu PA, Cowen EW.** 2012. Cutaneous graft-versus-host disease—clinical considerations and management. Curr Probl Dermatol **43:**101–115.

95. **Yamada K, Gianello PR, Ierino FL, Fishbein J, Lorf T, Shimizu A, Colvin RB, Sachs DH.** 1999. Role of the thymus in transplantation tolerance in miniature swine. II. Effect of steroids and age on the induction of tolerance to class-I-mismatched renal allografts. Transplantation **67:**458–467.

96. **Yamada K, Gianello PR, Ierino FL, Lorf T, Shimizu A, Meehan S, Colvin RB, Sachs DH.** 1997. Role of the thymus in transplantation tolerance in miniature swine. I. Requirement of the thymus for rapid and stable induction of tolerance to class-I-mismatched renal allografts. J Exp Med **186:**497–506.

97. **Yamada K, Ierino FL, Gianello PR, Shimizu A, Colvin RB, Sachs DH.** 1999. Role of the thymus in transplantation tolerance in miniature swine. III. Surgical manipulation of the thymus interferes with stable induction of tolerance to class-I-mismatched renal allografts. Transplantation **67:**1112–1119.

98. **Zhang L, Li Y, Jiang H, Liu J, Zeng Y, Cheng J.** 2005. Comparison of hepatic coagulant, fibrinolytic, and anticoagulant functions between Banna minipig inbred line and humans. Transplantation **79:**1128–1131.

99. **Zhang L, Li Y, Liu J, Zeng Y, Zeng R, Cheng J.** 2004. Activation of human coagulation system by liver-derived clotting factors of Banna minipig inbred line. Transplant Proc **36:**2490–2491.

100. **Zuckermann FA, Pescovitz MD, Aasted B, Dominguez J, Trebichavsky I, Novikov B, Valpotic I, Nielsen J, Arn S, Sachs DH, Lunney JK, Boyd P, Walker J, Lee R, Davis WC, Barbosa IR, Saalmuller A.** 1998. Report on the analyses of mAb reactive with porcine CD8 for the Second International Swine CD Workshop. Vet Immunol Immunopathol **60:**291–303.