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The Banff 2007 Working Classification of Skin-Containing Composite Tissue Allograft Pathology

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Composite tissue allotransplantation (CTA) is a recently introduced option for limb replacement and reconstruction of tissue defects. As with other allografts, CTA can undergo immune-mediated rejection; therefore standardized criteria are required for characterizing and reporting severity and types of rejection. This article documents the conclusions of a symposium on CTA rejection held at the Ninth Banff Conference on Allograft Pathology in La-Coruña, Spain, on 26 June 2007, and proposes a working classification, the Banff CTA-07, for the categorization of CTA rejection. This classification was derived from a consensus discussion session attended by the first authors of three published classification systems, pathologists and researchers from international centers where clinical CTA has been performed. It was open to all attendees to the Banff conference. To the extent possible, the format followed the established National Institutes of Health (NIH) guidelines on Consensus Development Programs. By consensus, the defining features to diagnose acute skin rejection include inflammatory cell infiltration with involvement of epidermis and/or adnexal structures, epithelial apoptosis, dyskeratosis and necrosis. Five grades of severity of rejection are defined. This classification refines proposed schemas, represents international consensus on this topic, and establishes a working collective classification system for CTA reporting of rejection in skin-containing CTAs.

Key words: Anitbody-mediated rejection, Banff, Banff schema, chronic rejection, composite tissue allograft, humoral rejection, rejection, skin allograft, transplant

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Background and Goals

Composite tissue allotransplantation (CTA) is an emerging discipline for the treatment of functionally significant tissue or limb defects. In contrast to solid organ transplants, CTAs often include skin as well as tissues of diverse embryological origin. Most CTA recipients have experienced reversible episodes of acute rejection (1) but to date, no universally accepted criteria for CTA rejection reporting has been established. Histopathology plays a key role in diagnosis of rejection, in understanding the physiopathology of rejection and in facilitating management. Currently, four classification systems have been published and as such, a universally accepted grading scheme for ranking pathological severity of rejection is needed. Standardization is necessary for reporting clinical results and to establish objective end points for clinical trials. Recognizing that a dispersed and unstandardized development of CTA would present a major barrier for progress and reporting, a collaborative relationship was established with investigators with experience in clinical CTA worldwide to initiate the groundwork for a universally accepted histological classification. In addition, as immunomodulatory regimens are minimized, CTA

will experience a growth period in the near future. This article describes a consensus schema for the stardardization of clinical reporting for the advancement of the study of the histopathology in CTA-containing skin for dissemination to the health care practice and medical community. As a working classification, the schema will continue to be refined in subsequent meetings as more clinical and experimental data become available for skin and other tissues used in CTAs.

Investigators in the field of CTA including representatives from multiple sites reporting a clinical CTA in the past decade were invited to a consensus discussion on CTA histopathology at the Ninth Banff Conference on Allograft Pathology. In keeping with established National Institutes of Health (NIH) guidelines on Consensus Development Programs (2), this conference included: (1) a broad-based nonadvocacy, independent panel gathered to give balanced, objective and knowledgeable focus to the topic, (2) freedom from scientific or financial conflict of interest from the speakers, (3) predetermined questions defining the scope and the direction of the conference, and (4) a systematic literature review of the topic. The presenters included the three first authors of the four classification systems published and investigators who have actively followed CTA patients from a clinicopathological view and/or published reports on CTA rejection. A pathologist from the center where the fourth classification system was published was also invited, provided a presentation and participated in the discussions. Six out of six western international centers with reported experience in hand transplantation at the time of the call were invited and five centers were represented. Furthermore, two out of two centers with experience in other CTA's-containing skin were represented (i.e. face and abdominal wall). All published scoring systems for CTA were reviewed (3–7). In addition, a senior investigator in CTA was invited to provide a historic perspective. Each presenter provided data followed by a discussion. Of the presenters, five were clinical pathologists, three were surgeons and one was a basic investigator. The session was open to the public and all attendees of the Banff Conference. A total of 20 attendees provided oral and/or written comments to the questions posed.

To date, 41 patients receiving skin-containing CTA's have been reported; 28 have received hands, three faces, one knee with a skin island and nine abdominal walls. Essentially, all patients have experienced episodes of rejection (1,8,10). Clinical manifestations of rejection have been characterized by cutaneous changes including mild pink discoloration, gradual erythema, macules progressing to red infiltrated lichenoid papules with or without limb edema and onychomadesis in advanced rejection (1,11). Histological findings disclose predominantly lymphocytic inflammatory-cell infiltrate of variable density, epithelial intracellular edema (spongiosis), lymphocyte exocytosis and keratinocyte apoptosis (1,12). Macroscopic skin changes in a case reported after steroid resistant rejection showed blisters in the superficial layers with epidermal desquamation. Histology revealed dermal and epidermal lymphocytic infiltration with apoptotic and necrotic keratinocytes (13).

The four published systems on grading CTA skin rejection ranked the degree of rejection based on evaluation of morphologic features (3–7). All systems illustrated substantial agreement on the basic grade stratification for acute rejection. All agreed that perivascular lymphocytic infiltrates become progressively denser and involve more vessels as the severity of rejection increases. The inflammation then extends to involve dermal stroma, epidermis (including the basal cell layer) and adnexa at moderate to marked grades of rejection. Epidermal apoptosis/necrosis is a marker of severe rejection in all of the published systems where it was observed. The classification based on full thickness, vascularized, myocutaneous-free flaps for closure of abdominal defects (3) stratified rejection based on the extent of vessels infiltrated, from $<10\%$, to 11–50% in mild, and to more than 50% in moderate and severe rejection. Severe rejection of abdominal wall grafts showed dyskeratosis and spongiosis.

The discussion initiated with the following predetermined questions chosen by the CTA session committee chair in conjunction with investigators in the field: (1) Specimen and Slide Preparation: which structures are required to constitute an adequate sample? How will the biopsy be taken to appropriately reflect the clinical involvement? How many samples are required? What are the stains besides hematoxylin and eosin (H&E) that should be applied? (2) Scope of disease-acute: What are the basic features to diagnose rejection? What other features should be recorded and how? What should be excluded from acute rejection? (3) Lesion scoring-acute: How will severity be graded? (4) Scope of disease-chronic: What are the defining features of chronic injury? (5) Scope of disease-humoral: What information should be collected to define this effector mechanism in CTA?

The questions were provided to the participants in both oral and written formats. Oral and written comments were collected throughout the consensus discussion session. This article represents the recompilation of the discussions including all oral and written comments.

Specimen Adequacy

Allografts that include skin are distinctive in that rejection can be recognized by visual inspection. To include this unique feature of CTA, the clinical involvement as assessed visually at the time of biopsy or rejection should be reported as no visible changes, $<10\%$, 10–50% and $>50\%$ of the CTA. Features include but are not limited to rash, edema, erythema, vesiculation, desquamation, necrosis and/or ulceration. To diagnose and classify skin rejection, specimen adequacy is defined as at least one 4-mm punch biopsy

taken from the most reddened and/or indurated but apparently viable area of involved skin. Only one biopsy is required for diagnosis, to avoid unnecessary scarring, especially with multiple episodes of rejection. The structures required to constitute an adequate sample are the epidermis and its adnexa, dermis, subcutaneous tissue and vessels. The recommendations for slide preparation are hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) stains. Immunohistochemical stains are also recognized as potentially important and are thus recommended "as needed" based on H&E findings and/or for research purposes. These included but are not limited to CD3, CD4, CD8, CD19, CD20 and CD68, as well as HLA-DR, CMV and C4d. The use of trichrome stain is not considered mandatory at this time but could be performed if desired.

Acute Cell-Mediated Rejection

The basic features to diagnose and classify rejection requiring specific comment in diagnostic reports are immune cell infiltration, and epidermal and/or adnexal involvement namely spongiosis, apoptosis, dyskeratosis and necrosis. The cellular infiltrate can be mixed (e.g. including neutrophils) and not limited to lymphocytes. The pattern of the infiltrate should be characterized as perivascular or interstitial, focal or diffuse and dermal and/or hypodermal. Early signs of rejection may include the presence of scattered dermal infiltrates. Interface inflammation/dermatitis is an important feature to identify, as this may relate to the severity of the rejection or may signal a nonrejection etiology. Infiltration of eosinophils should be recorded descriptively but is not included in the current classification. This will allow the study of its significance in the future.

As in other pathologies in which ulceration or necrosis develops, vasculitis may be either primary or secondary to the ulceration. Indications of rejection-related vasculitis include: absence of a history of trauma; involvement of vessels distant from the ulcer; multi-focality of the necrotizing process within the affected vessel; and involvement of several vessels within the biopsy, particularly vessels of various sizes and depths within the dermis. The pathologic and clinical features of immune and nonimmune processes are potentially overlapping and will require further study. Because there is insufficient data to absolutely exclude nonimmune conditions from a particular CTA biopsy, a descriptive observation is currently the appropriate format for reporting findings. As with solid organ transplants, other inflammatory, infectious or neoplastic processes may coincide with acute rejection.

The Banff CTA 2007 Classification for Cell-Mediated Acute Rejection of Skin

The acute/active skin rejection scoring system was divided in five grades, based on intensity and localization of infiltrates. The rejection classification is shown in Table 1.

Chronic Rejection

Currently, insufficient data are available to define specific changes of chronic rejection in a CTA. Chronic changes and injury to an allograft evolve over time with persistent immune insult and are likely to be altered in tempo and character by concomitant treatment. Fibrosing changes can also be caused by nonimmune events, and in certain circumstances both can overlap. Histologic and clinical features highlighted as indicative of chronic injury in a CTA include vascular narrowing, loss of adnexa, skin and muscle atrophy, fibrosis of deep tissue, myointimal proliferation and nail changes. As with other solid organs, it is likely that chronic/persistent injury begets a common histological phenotype through a variety of nonexclusive mechanisms. A possible correlation between graft-versus-host disease (GVHD) and CTA-skin was noted.

Antibody-Mediated Rejection (AMR)

There is not enough information to draw any conclusions regarding AMR. However, several pieces of histologic and clinical information should be gathered in order to define AMR in CTA. These include the presence of C4d deposition and its relationship with donor-HLA-specific antibodies as well as the presence of vasculitis, neutrophilic margination, thrombi and necrosis. A complete history including patient sensitization (e.g. PRA, cross-match results, transfusions, pregnancies and previous allografts) as well as the presence or absence of autoantibodies and T- and B-cell crossmatch is to be performed before transplantation. The correlation between graft dysfunction and rejection in CTAs has not been established. Therefore, clinical evidence of graft dysfunction is not included at this point.

Table 1: The Banff 2007 working classification of skin-containing composite tissue allograft pathology

Grade 0. No or rare inflammatory infiltrates.
Grade I. Mild. Mild perivascular infiltration. No involvement of the overlying epidermis.
Grade II. Moderate. Moderate-to-severe perivascular inflammation with or without mild epidermal and/or adnexal involvement (limited to spongiosis and exocytosis). No epidermal dyskeratosis or apoptosis.
Grade III. Severe. Dense inflammation and epidermal involvement with epithelial apoptosis, dyskeratosis and/or keratinolysis.
Grade IV. Necrotizing acute rejection. Frank necrosis of epidermis or other skin structures.

Table 2: Differential diagnosis in skin allograft biopsies

Infections
Posttransplant lymphoproliferative disorder (PTLD)/lymphoma
Insect bites
Drug reactions/toxicity
Eosinophilic dermatitis
Graft vs. host disease
Allergic or irritant contact dermatitis
Other

Related/Nonrejection Pathology

It was recognized that skin changes in a CTA are not limited to alloimmune injury (8). Specific differential diagnoses to consider include; infections (particularly fungal); drug toxicity (e.g. topical steroids or other drugs); posttransplant lymphoproliferative diseases (PTLD)/lymphoma; insect bites; GVHD; allergic or irritant contact dermatitis and eosinophilic cellulitides (see Table 2). Detailed description of these processes are beyond the scope of this manuscript, but should be identified in reporting CTA pathology.

Observations

With this international effort, we have initiated an international consensus approach that will progress over time. Standardized reporting of results should advance research related to CTA. Common methods of data collection facilitate clinical interpretation, communication between clinicians and pathologists and prospective data compilation for future studies. As a working classification, the schema will continue to evolve and develop as more scientific information becomes available for skin and other tissues included in CTA.

This new international classification follows the published systems, which adopted a tiered approach to grading rejection. Tiered systems tend to differentiate levels of severity by the addition of a new lesion at each level. In this classification, the first lesion to appear is perivascular inflammation, which is usually mild and focal. In grade II lesions there is expansion of the infiltrate accompanied by involvement of epidermis or adnexa but without dyskeratosis or epidermal apoptosis. Grade III adds these latter features of individual cell injury, while grade IV adds frank necrosis.

Both dermal edema and spongiosis reaction are found in a wide range of disorders including those associated with immunologic abnormalities, infections and neoplasia. Some form of microvascular injury in the dermis is thought to be the initiating phenomena withdermal fluid transiting to the epidermis producing spongiosis. Outside the field of transplantation, cell-mediated and antibody-mediated processes have been implicated in the reaction along with other causes of endothelial/vessel wall injury. Edema (dermal and epidermal spongiosis) is likely to be part of many reactions in the allograft and is probably nonspecific, though the severity and extent of edema may assist in the interpretation of the process. However, further studies are needed in this area.

Consideration was given to the histopathology of cutaneous GVHD as a parallel to alloimmune injury in skin-containing CTA. In 2006, the National Institutes of Health Consensus Development Project Pathology Working Group presented requirements for the diagnosis of chronic GVHD (14). The report presented the progression of histologic changes from acute-to-chronic cutaneous GVHD including four chronic forms: skin lichen planus-like, sclerotic, morpheic and fascitis. The sclerotic stage is characterized by a zone of relatively avascular collagen, which replaces the papillary and upper reticular dermis. Microscopically, there is hyperkeratosis with flattening of rete ridges, vacuolar changes of the basal cell layer and lymphocytic infiltration and epidermal melanin incontinence. The report highlighted the histologic changes in chronic GVHD related to immunosuppressive therapy and underlined the need for studying the significance of perivascular lymphocytic inflammation or persistent vacuolar degeneration after treatment. It is possible that chronic skin changes in CTA might parallel those of chronic cutaneous GVHD and 'chronic rejection' in other types of transplants. Clinicopathologic correlation from prospective data will aid in the assessment of chronic changes in CTA not only for skin but for all tissues involved in a CTA.

The implications of several pathological changes unique to limb transplantation remain to be determined. These include changes associated with the nail bed, which have been suggested to be evidence of chronic persistent immune injury but could also be precipitated by more acute inflammatory invasion of the nail matrix. While the Banff CTA 2007 scoring system focuses on the rejection-related changes, there are a number of other immune and nonimmune mediated processes that must be recognized and contemplated in the differential diagnosis (Table 2) (8).

The Banff CTA 2007 grading scheme for acute rejection is developed enough to have immediate clinical utility. It is likely that, similar to solid organ transplantation, CTAs will undergo indolent chronic changes and a grading of severity of chronic rejection will evolve in this working classification. CTAs that contain skin are unique in that rejection-related changes can be directly observed. Indeed, it has been shown that significant perivascular infiltration appears coincident with a skin rash (13,15–16). Areas of scleroderma resulting from chronic infiltration and injury have been noted at times in the absence of significant infiltrates (Charles W. Hewitt, personal communication, La Coruña, Spain, 26, June 2007). However, given the heterogeneity of CTAs, and the potential that repeated

inflammation could manifest as chronic dysfunction, it is important to begin cataloging the histology of CTA rejection in objective terms. The visualization of skin changes can be used as a clinical indicator of rejection; however, the sensitivity and specificity of rash and/or other sign as markers of rejection remains to be established, as does the evidence of histological response to therapy. To this end, the clinical percentage of gross graft involvement has been added as a starting point for correlation between clinical presentation and severity of rejection.

The fundamental biology underlying CTA is sufficiently similar to that of other solid organs that additional phenotypes of rejection will appear as the clinical experience grows, including AMR, chronic fibrosis/atrophy and vascular rejection. These will be addressed at subsequent congresses and characterized based on accumulated experience in the field. As an emerging field, many questions remain unanswered, and there is ample opportunity for clinical and basic investigation. Future directions include the characterization of the infiltrating cells and their function, the study of accommodation, chronic injury and AMR, the utility of molecular studies and the inflammatory response in this complex transplant. This Banff CTA-2007 classification is an international effort to lay the groundwork to advance the understanding of CTA pathology. It will enhance the communication between investigators and will contribute to clinical analysis.

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