Mucosal vascular alterations in isolated small-bowel allografts: relationship to humoral sensitization

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Abstract

Acute vascular rejection (AVR) in human small-bowel transplantation is an inadequately characterized entity whose frequency and severity is not well understood. As compared to severe AVR, changes identifying early, mild or evolving AVR are not known. We created a scoring system to evaluate subtle mucosal vascular changes and examined 188 biopsies from 21 patients obtained in the first 3 months post transplant. A majority of patients had a transient rise in vascular injury, often within 30 days of transplant. Small-vessel congestion and erythrocyte extravasation were the most common alterations. The vascular injury score was not related to acute cellular rejection, HLA type or HLA antigen disparities. However, the patients with the vascular changes had significantly higher peak panel reactive antibodies (PRA) and a higher incidence of positive T-cell and B-cell crossmatch. Finally, graft survival was significantly lower in the patients demonstrating the early vascular lesions. These data suggest that the vascular injury is partially associated with humoral presensitization of the recipient and may be a form of acute [...]
Mucosal Vascular Alterations in Isolated Small-Bowel Allografts: Relationship to Humoral Sensitization

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Acute vascular rejection (AVR) in human small-bowel transplantation is an inadequately characterized entity whose frequency and severity is not well understood. As compared to severe AVR, changes identifying early, mild or evolving AVR are not known. We created a scoring system to evaluate subtle mucosal vascular changes and examined 188 biopsies from 21 patients obtained in the first 3 months post transplant. A majority of patients had a transient rise in vascular injury, often within 30 days of transplant. Small-vessel congestion and erythrocyte extravasation were the most common alterations. The vascular injury score was not related to acute cellular rejection, HLA type or HLA antigen disparities. However, the patients with the vascular changes had significantly higher peak panel reactive antibodies (PRA) and a higher incidence of positive T-cell and B-cell crossmatch. Finally, graft survival was significantly lower in the patients demonstrating the early vascular lesions. These data suggest that the vascular injury is partially associated with humoral presensitization of the recipient and may be a form of acute vascular rejection. Since these vascular changes are frequent, we advocate early post-transplant monitoring to identify and manage potentially high-risk patients.

Key words: Small bowel transplantation, vascular rejection

Received 19 June 2002, revised and accepted for publication 29 August 2002

Introduction

Human small-bowel transplantation has become a mainstay for the treatment of intestinal failure (1–3), particularly in the situation where extended usage of total parenteral nutrition (TPN) has caused significant morbidity in the patient. The success of bowel transplantation has markedly improved with the employment of modified immunosuppressive protocols that have included reagents such as tacrolimus, and refined surgical techniques (2,3). However, several factors remain as significant impediments to optimal graft survival. Notable among these obstacles is the continued presence of acute rejection, infections, post-transplant lymphoproliferative disease and occasional graft vs. host disease (4–6).

Acute rejection in the bowel allograft appears to originate in similar fashion to that in other solid organ allografts, with primary involvement of the cell-mediated and humoral-based arms of the immune response (7,8). When recognized in the early stages of involvement in the bowel transplant, acute rejection can often be successfully managed through an appropriate adjustment of immunosuppression. On occasion, however, therapy-resistant acute rejection is present which requires more aggressive immunosuppressive intervention.

At this point, the identification of acute rejection in bowel is accomplished by considering a constellation of findings, including clinical symptoms (e.g. increased ostomy output), endoscopy appearance and histopathological changes seen in the mucosal biopsy (9). The endoscopically obtained biopsy is typically considered the most accurate appraisal of the rejection process, but with the caveat that there is an adequate amount of tissue for assessment (10). The small size and the restriction of the biopsy to the mucosal component of the bowel impose some limitations in the evaluation of processes occurring in deeper layers of this organ. Despite these limitations, a reasonable appreciation of the histopathological alterations associated with acute cellular rejection (ACR) in bowel has been attained (9).

As with other solid organ allografts (11), the inflammatory changes of acute cellular rejection in the bowel principally involve the interstitial region of the organ, with infiltration and cytopathic effects on surrounding epithelial cells comprising crypt and villous structures (12). T lymphocytes are the principal immune effector cell population involved in ACR (13). By comparison, acute vascular rejection (AVR) in bowel transplants remains insufficiently described, particularly, in scenarios where there may be low to moderate levels of this form of rejection. Severe AVR in the bowel does appear to have several prominent characteristic features, but this is an uncommon, albeit destructive entity (14). Therefore, it is likely that milder forms of AVR are not being suitably assessed in mucosal bowel biopsies due to our lack of understanding of their frequency and the associated morphological alterations. In this regard, we hypothesized, based on our and others’ previous experience with other solid organ allografts, that subtle morphological changes occur in the microvasculature of the bowel mucosa during the course of an ensuing or mild acute vascular rejection. We developed a grading scheme for evalu-
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altering vascular changes, and present our scoring results herein for a series of SBTx biopsies as well as concomitant clinical features, including evidence of humoral sensitization.

Materials and Methods

This was a retrospective study of biopsies from small-bowel transplants performed at the University of Miami Department of Surgery, Division of Transplantation, from 1999 to 2001. The period of study was limited to the first 3 months post transplant. Consent for possible inclusion in the study was obtained prior to transplantation. Isolated small-bowel transplantation was performed in accord with established techniques (15).

Venous drainage was supplied either via the portal vein or by anastomosis to the inferior vena cava. All patients had distal ileostomies created for accurate volume measurement and to provide access for small-bowel biopsies. Immunosuppression was based on tacrolimus (prograf, Fujisawa) and steroids. A variety of demographic and clinical variables were studied and assessed for their association with the pathologic alterations. Clinical suspicion of acute rejection was included as a variable for comparison.

Pathology

Mucosal biopsies were obtained via endoscopic procedures, and the tissue was immediately placed in a 10% buffered formalin solution for no less than 2 h. The biopsies were then routinely processed using a formalin-based tissue-processing method and embedded in paraffin. The sections were then cut at 5.0 μm and stained with Hematoxylin and Eosin (H&E) and with Trichrome. Acute cellular rejection was evaluated and assigned a numerical score of 0–4 for each of the overall diagnostic categories. (0 = normal; 1 = indeterminate for acute rejection; 2 = acute cellular rejection, mild; 3 = acute cellular rejection, moderate; 4 = acute cellular rejection, severe).

A semi-quantitative scoring system was also developed for appraising subtle vascular alterations in the intestinal mucosa (Table 1). Capillaries and small venous and arterial branches in the lamina propria and the submucosa were evaluated for the presence of dilatation and erythrocyte congestion. In addition, the surrounding interstitium was assessed for the presence of extravasated erythrocytes and edema. Scoring was calculated and based on the percentage of the overall biopsy (for the ‘general score’) of the specific region (villous vs. mucosal) which demonstrated vascular congestion or RBC extravasation.

Table 1: Semi-quantitative scoring system for the evaluation of vascular changes in small bowel allograft mucosal biopsies

<table>
<thead>
<tr>
<th>Grade</th>
<th>Histopathological alterations(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No significant congestion or extravasation</td>
</tr>
<tr>
<td>1</td>
<td>10–40% of the tissue shows changes</td>
</tr>
<tr>
<td>2</td>
<td>40–70% of the tissue shows changes</td>
</tr>
<tr>
<td>3</td>
<td>70% or greater portion of the tissue shows changes</td>
</tr>
</tbody>
</table>

\(^1\)Capillaries and small venous and arterial branches in the lamina propria and the submucosa were evaluated for the presence of dilation and erythrocyte congestion. In addition, the surrounding interstitium was assessed for the presence of extravasated erythrocytes and edema. Scoring was calculated and based on the percentage of the overall biopsy (for the ‘general score’) of the specific region (villous vs. mucosal) which demonstrated vascular congestion or RBC.

Immunohistochemical staining

Tissue from several patients (n = 5; three with vascular changes, two with no vascular changes) was simultaneously obtained and frozen for immunohistochesmical studies. The tissue was snap-frozen in OCT and cut on a cryostat at 4 μm sections and slides were fixed in acetone. Slides were washed in PBS and direct immunofluorescence staining was performed on the tissue, using several antibodies that included fluorescein-conjugated anti-human IgG, -IgA, -IgM, -C3 and -C4 (BioWhittaker, Walkersville, MD, USA). The antibodies were incubated for 30 min at room temperature, and then washed twice with PBS. In the case of C4d, indirect immunofluorescence staining was performed after the initial wash step by incubating the slides with an unlabeled primary antibody (mouse anti-human C4d monoclonal antibody) (Quidel, San Diego, CA, USA) for 60 min at room temperature. These slides were washed twice with PBS and then incubated with fluorescein-conjugated goat anti-mouse IgG (ICN Pharmaceuticals, Costa Mesa, CA, USA) for 30 min, at room temperature. The slides for both the direct and the indirect procedures were washed after the last antibody incubation and cover-slipped. After mounting, the slides were observed with a Leica Laborlux S fluorescence scope (Leica, NuBloch, Germany).

HLA typing, panel reactive antibodies (PRA) and crossmatching

A determination of HLA cell-surface antigens was accomplished by a complement-dependent microlymphocytotoxic technique (16), using a commercial assay kit (One Lambda, Canoga Park, CA, USA). The screening of sera for class I and class II antibodies was performed with a complement-dependent microlymphocytotoxic technique, using a commercial assay kit (Lambda Cell Tray; One Lambda, Canoga Park, CA, USA). Crossmatching of recipient serum with donor cells at the time of transplant was accomplished as previously reported (17).

Statistics

P-values were obtained for comparison of mean values within the different experiments using Student’s paired t-test.

Results

We evaluated a series of sequential biopsies from patients with small-bowel allografts during the initial 3 months of the post-transplant period. This time period can demonstrate AVR due to prior sensitization and/or de novo development of effector arms of the immune response. We found that shortly after transplantation, a notable proportion of biopsies demonstrated vascular alterations in the mucosal layer of the bowel. Figure 1 demonstrates examples of the range and relative scores we assigned for these changes. The changes ranged from mild vascular congestion in a small portion of the biopsy vs. ubiquitous, significant congestion and erythrocyte extravasation. As shown in Figure 2, the highest scores were attained at 10 days after transplant, followed thereafter by a gradual decrease in the vascular score. By 50 days post transplant, the overall vascular score reached its nadir and then began to rise once again. The villous region and lamina
Vascular Changes in Small Bowel Allografts

Figure 1: Representative photomicrographs of human small-bowel allograft with varying degrees of mucosal vascular injury, particularly, vascular congestion and red cell extravasation. Upper left: grade 0, no significant changes; upper right: grade 1, mild vascular injury; lower left: grade 2, moderate vascular injury; lower right: grade 3, severe vascular injury. Hematoxylin and eosin, 200x.

Propria were the areas principally affected by these changes, although separate scoring of this latter area vs. the crypts revealed similar patterns for specific pattern scoring, as evident from the overall scores shown in Figure 2 (data not shown). There was no evidence of any significant vasculitis in the biopsies we evaluated. Measurements of acute cellular rejection scores showed variable patterns and time courses with no statistical association with the vascular changes we observed (data not shown).

We evaluated a variety of demographic features of our patient populations, including the HLA types and disparities (between donor and recipient), immunosuppressive therapies, age, sex, original disease, and did not find any association with vascular changes in the bowel mucosa. We then evaluated whether there was any evidence of humoral sensitization in the patients before the transplant that could have some association with the observed vascular changes. Figure 3 shows that the percentages of panel reactive antibodies...
to class I and II antigens at the time of transplant were highest in those patients who had the highest vascular scores. Of the patients with PRA, only one had a sensitization to antigens present on the donor (data not shown); this particular patient had a moderate amount of vascular injury present. Furthermore, there was a higher proportion of patients with positive crossmatches at the time of transplant in those patients with a vascular score above 1 in the initial months post transplant (Figure 4).

Graft survival was calculated, and our results indicated that a statistically significant increased number of patients with higher vascular scores also lost their grafts due to rejection (Figure 5). Finally, we were able to perform immunostains on several patients who had enough tissue for parallel evaluation. We found C4d, IgM and IgG deposition (an example is shown in Figure 6) in the three patients with the vascular changes. The C4d was variable in intensity and involved the small vessels and arterial branches. Of the patients with no significant vascular alterations, one had trace C4d and immunoglobulin staining and the other patient appeared negative.

Discussion

In this study, we have demonstrated that during the initial months post transplant a considerable proportion of small-bowel allografts exhibit alterations of the bowel mucosa that suggest congestion and possible injury to the microvasculature. These changes can be subtle and required the establishment of a semi-quantitative grading scheme in order to discern whether there were distinguishable variations over the course of the transplant period. Using this scoring system, we found that these congested vessels and areas with erythrocyte extravasation, when present, were most prominent in the initial weeks following transplantation. Moreover, the vessels of both the mucosa and submucosa appeared similarly affected, thus indicating that a measurement of these alterations in the portion of the intestine obtained by the endoscopically derived bowel biopsy can be representative of vascular changes occurring throughout the organ. With time, these vascular changes tended to attenuate in intensity and frequency, so that only a minor number of cases revealed these changes by 3 months after transplantation.

Our data raise the prospect that the etiology of this vascular injury could at least partially be due to an ongoing acute vascular rejection. This is most strongly supported by the findings that a notable proportion of the patients with these
Vascular Changes in Small Bowel Allografts

Figure 6: Immunofluorescence photomicrographs (all 200×) of human small-bowel allograft that had demonstrated significant mucosal vascular injury. Top photo shows C4d deposition on a small artery, middle photo shows C4d deposition in smaller vessels and capillaries, and bottom photo shows IgG deposition in a small artery.

Changes also had a positive antibody crossmatch and a propensity for heterogeneous antibody production (i.e. higher titer of panel reactive antibodies at the time of transplant). Moreover, four patients who had sufficient tissue for analysis showed the deposition of C4d and immunoglobulin on small vessels. Unfortunately, the limited amount of tissue typically provided in the biopsies prevents a consistent immunohistochemical assessment of the tissue, although our future studies will adequately address this issue. The temporal appearance of the vascular changes, along with the aforementioned clinical findings and with our observation that these patients often responded to additional immunosuppressive treatment, lend credence to the consideration that these alterations in vessels may be a manifestation of acute vascular rejection. The mild increase in the later time post transplantation could represent a recurrence of the acute vascular rejection or in some cases, de novo alloantibody production. Some cases with this later increased vascular change were considered acute rejection and were successfully managed with an increase of immunosuppression.

There are several other possibilities for the origin of this vascular injury, one being ischemia–reperfusion (IR) injury (18) in the bowel. However, the highest frequency of vascular changes occurring in the initial weeks post transplant is not ideally compatible with an IR effect, since one would expect an earlier time point for peak injury. Moreover, we did not see any significant incidence of other changes more typically ascribed to IR injury in bowel allografts, such as focal detachment and edema of the superficial villi and increased amounts of neutrophils. We also did not observe any significant differences in the ischemia time or surgical complications with those patients who had the vascular changes. These data taken together lessen the likelihood of IR injury as an underlying cause of this vascular affect. Other potential origins for the vascular changes could include transient vascular pressure differences (e.g. between donor–recipient vessels), infections, vasocclusion/ischemia and drug effects. While these latter entities should be considered, we did not find any significant clinical, radiological or pathological evidence to support them as a cause of the vascular injury.

The morphological evidence of acute vascular rejection in other solid organ allografts is varied but includes changes such as arterial vasculitis, neutrophilic accumulation and margination, vascular congestion, thrombosis and hemorrhage, and other changes in nonvascular structures such as glomeruli (19). The intensity and pattern of arterial vasculitis varies significantly among different forms of AVR and depends upon the transplanted organ and whether the biopsies are from clinical or experimental studies. In fact, kidney allografts show a gradation of vasculitis from minimal inflammatory changes to circumferential fibrinoid necrosis, and this forms the basis of classification of the humoral forms of acute rejection in the Banff Scheme (11). The description of AVR in bowel has generally been limited to examples of severe cases with widespread and dramatic morphologic alterations with a fulminant clinical course. Otherwise, our knowledge of the histopathological alterations in bowel allografts undergoing acute rejection with a vascular component comes from experimental animal models (20). Adjunct findings in various forms of AVR can include immunohistochemical or molecular evidence of antibody within the graft, especially directed to vessels and preferably donor specific (21). Recent studies in kidneys have further suggested that one byproduct of the
antibody-mediated immune response, namely C4d, is covalently bound to some vessels after complement fixation, and its detection in peritubular capillaries of renal tissue is an indicator of a humoral based immune response (22). In this regard, our initial findings of C4d deposition lend credence to the notion that our observed morphological changes are related to the presence of alloantibody. Our findings then raise the possibility that acute vascular rejection is present at a higher frequency in this type of solid organ transplantation, although likely in milder and more treated forms. The reasons for this are unknown, although the bowel could have a higher incidence of AVR (albeit milder), with the large antigenic load and peculiar cellular constitution of this organ allograft probably playing a role.

It is generally accepted, based on numerous animal models and clinical studies, that antibodies directed to donor antigens (i.e. humoral response), could initiate and maintain acute vascular rejection (21), depending upon the type and level of the antibody, as well as the type of transplant scenario. However, it is also clear that there can be a T-cell-mediated vasculitis occurring in allografts (23,24), therefore invoking the cell-mediated arm of the immune response. Likely, the most important determinant, aside from which effector arm of the immune response is involved in AVR, is that the predominant (and probably, most important) target in this form of rejection is the endothelial cell (25). This cell type, when besieged by recipient immune pathways, becomes involved in the complex cascades and pathways that predominate during a vigorous alloimmune response. It is thus understandable that immune-mediated injury to this cell type manifests during the acute phase of the type of rejection with vascular morphologic alterations. Ultimately, prolonged injury to the endothelial cell also is likely a crucial step in the development of chronic rejection (26).

A credible speculation, based on these studies, is that in the case of bowel allografts, presensitization of donor-specific humoral pathways can result in the changes we observed. In cases where there is no firm evidence of pretransplant humoral sensitization, there is also the possibility that de novo generation of donor-specific alloantibodies may be contributing to the vascular alterations and those sequelae. Furthermore, as noted above, the T-cell-mediated response to endothelial cells of the graft may be participating in the AVR. While vasculitis can be a cornerstone histopathological finding in severe AVR, this is not the case in less forms of this type of rejection (11). Considering the clinical evidence for our patients being at risk for humoral sensitization with the finding that we did not see overt vasculitis in our studies raises the possibility that these vascular alterations could be representing less severe AVR that is being successfully managed in the face of significant immunosuppression. In some cases, it may be that the AVR remained at a clinically quiescent level, but this could still be contributing to reduce graft survival in those patients who had the more significant vascular injury.

In summary, we feel that a recognition and grading of the mucosal vascular alterations in bowel allografts during the initial months of the post-transplant period may be a useful marker for identifying patients at increased risk of losing their grafts. The mechanism of these vascular changes may be the result of an acute vascular rejection. If acute vascular rejection is the underlying cause for these changes, then the potential for a resistant rejection has to be considered; this also makes the clinician aware that alternative therapies (e.g. plasmapheresis) may be ultimate issues to consider for the patient. Studies are ongoing to further address and characterize the mechanisms for these changes (e.g. which immune mechanisms may be participating) as well as the utility of altered immunosuppressive regimens that are applied when this vascular injury is identified.

Acknowledgments
The authors wish to thank D. Mero for clerical assistance.

References
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