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CASE REPORT

Graft vasculopathy in the skin of a human hand allograft: implications for diagnosis of rejection of vascularized composite allografts

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Summary

Whereas vascularized composite allografts often undergo acute rejections early in the postgraft period, rejection manifesting with severe vascular changes (graft vasculopathy) has only been observed on three occasions in humans. We report a hand-allografted patient who developed severe rejection following discontinuation of the immunosuppressive treatment. It manifested clinically with erythematous maculopapules on the skin and pathologically with graft vasculopathy that affected both large vessels and smaller cutaneous ones. The observation that graft vasculopathy can affect skin vessels shows that it is amenable to diagnosis with usual skin biopsy as recommended for the follow-up of these allografts. Graft vasculopathy developing in the setting of vascularized composite allografts likely represents chronic rejection due to under-immunosuppression and, if confirmed, should be included in a future update of the Banff classification of vascularized composite allograft rejection.

Introduction

Following the human hand allograft (HHA) performed in 1998 [1], which was the first modern era skin-containing vascularized composite tissue allograft (VCA), several other VCA were performed successfully worldwide in humans, including mainly hands/forearms, arms, face, and abdominal wall. The largest experience concerns HHA, of which 68 cases have been so far performed [2]. VCA often undergo in the early postgraft period episodes of acute rejection (AR). These manifest with maculopapular or diffuse erythema of the allografted skin and comprise pathologically dermal and/or epidermal changes such as perivascular lymphocytic infiltration and epidermal necrosis/apoptosis [3], representing the criteria of the Banff 2007 classification of VCA rejection [4]. With the increasing number of VCA performed, new pathologic changes in VCA were subsequently observed, such as dermal capillary microthrombosis [5]. Rare cases of severe rejection leading to allograft loss showed vascular changes similar to those observed in chronically rejecting renal and cardiac allografts, known as "graft vasculopathy" (GV). GV is characterized by myointimal proliferation and fibrosis of blood vessels, resulting in vascular narrowing and occlusion, leading to ischemia and

organ dysfunction. So far three cases of GV have been observed in human VCA, including one knee [6] and two HHA [7], affecting mostly deep arteries. We report a new case of GV observed in a rejecting HHA that affected also medium-sized vessels of the skin. The pathogenic and diagnostic implications of this observation in human VCA are discussed.

Case report

A 35-year-old Italian man received on October 17, 2000 a right-hand allograft (wrist level). There were six HLA donor/recipient mismatches. The induction therapy included basiliximab, mycophenolate mofetil (MMF), tacrolimus and steroids and the maintenance one tacrolimus, MMF, and prednisone. The patient developed postgraft diabetes mellitus, CMV infection, bacterial infection of the connective tissue of the allograft, and temporary increase in creatinine values. He developed several AR episodes (on postoperative days 76, 2653, 4400, and 4500) that were reversed with intravenous steroids and topical tacrolimus ointment. Donor-specific antibodies (DSA) were checked at each anniversary of transplantation (until 2011) but were never detected. On day 4600, AR was again diagnosed, but the patient refused additional treatment, and admitted he had tapered his immunosuppressive treatment against medical advice over the last year. Diffuse erythematous maculopapules containing crusted necrotic lesions were seen over the allograft skin (Fig. 1). The allograft was removed on day 4680 (i.e., 13 years postgraft), while the patient had been reportedly off immunosuppressive treatment for 1 month. Tissue specimens were obtained from the amputated allograft and studied with routine histology (formalin-fixation, paraffin-embedding, staining of 4-micron-thick sections with hematoxylin–eosin–saffron) and immunohistochemistry as described previously [8]. They included the radial artery (at the wrist level), skin (from the volar and dorsal hand), muscle, and nerve.

The radial artery showed microscopically severe changes suggestive of GV, that is, an important fibrous thickening of the wall with lymphocytic infiltration, focal calcification, and fibrinoid necrosis (Fig. 2a and b). The inner elastic lamina was focally disrupted (Fig. 2c). Changes in GV were also found in vessels surrounding the radial nerve (Fig. 2d). No notable changes were seen in the muscle, bone, cartilage, and tendon (save a moderately dense perivascular lymphocytic inflammation in the latter).

Both skin specimens showed severe changes, including some that are seen in Banff grade II rejection, but also with additional findings not included in the current (2007) Banff classification. The dermis contained a dense perivascular infiltrate mostly consisting of $CD3^+/CD4^+$ T cells, with fewer amounts of $CD8^+$, TIA-1^+ cytotoxic and $FoxP3^+$ T-reg cells, some $CD20^+$ B cells, and $CD79a^+/CD138^+$ plasma cells. The epidermis showed no inflammation, dyskeratosis/

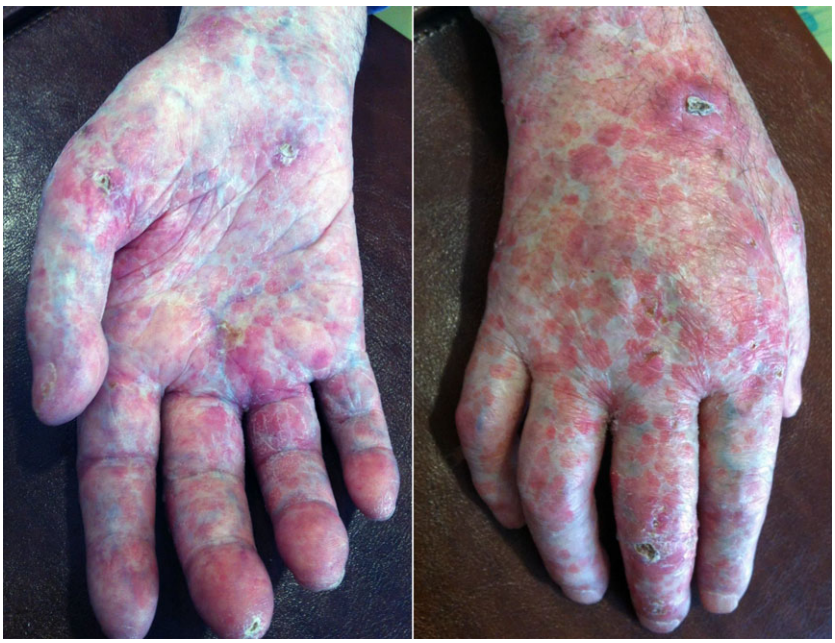

Figure 1 Clinical appearance of the allograft prior to amputation: diffuse erythematous cutaneous maculopapules containing some crusted necrotic erosions on the dorsal hand and the palm.

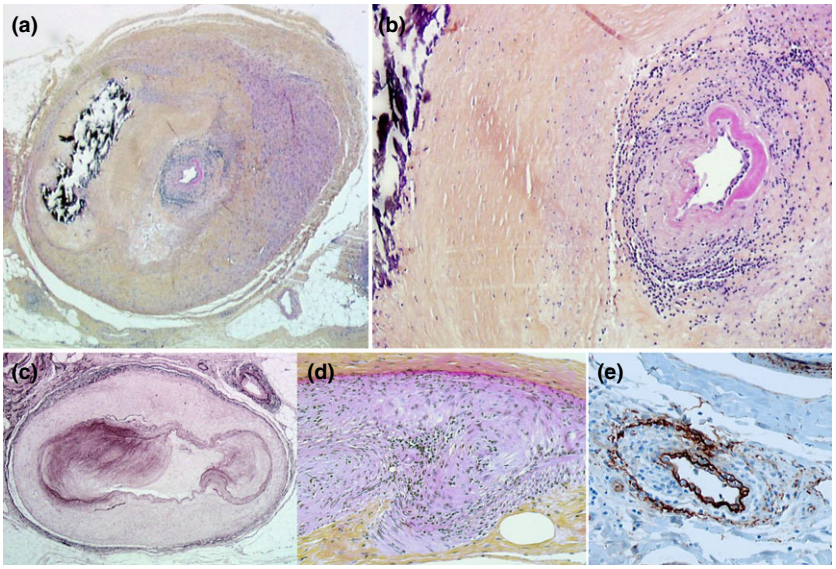

Figure 2 (a) Severe changes suggestive of graft vasculopathy in the radial artery: fibrous thickening and calcification of the wall, focal fibrinoid necrosis, highly narrowed lumen. (b) close-up view of panel a. (c) orcein staining shows focal disruption of the inner elastic lamina. (d) myointimal thickening of a perineural vessel. (e) C4d deposits on endothelial cells of a perineural vessel.

apoptosis, or necrosis. The most remarkable changes were found in the mid- and deep dermis and concerned medium-sized arterioles. These had highly thickened walls due to intimal hyperplasia, and narrow, occasionally occluded lumina (Fig. 3a and b). Endothelial cells were often swollen. The wall of several of the affected vessels was densely infiltrated mostly by $CD3^+/CD4^+$ T cells (Fig. 4a and b), with fewer amounts of $CD8^+$ T cells (Fig. 4c), $CD20^+$ B cells (Fig. 4d), and $CD79a^+/CD138^+$ plasma cells (Fig. 4e), admixed with some TIA-1^+ cytotoxic T cells and $FoxP3^+$ T-reg cells (Fig. 4f). These cells expressed HLA-DR antigens, also expressed by endothelial cells of the affected vessels (Fig. 3g). Dermal capillaries in the papillary dermis were thickened and had narrow lumina.

Immunoperoxidase staining for C4d, performed as described previously [8], showed deposits on rare perineural vessels (Fig. 3e) but not in those of the skin (Fig. 4h), the muscle, or the radial artery.

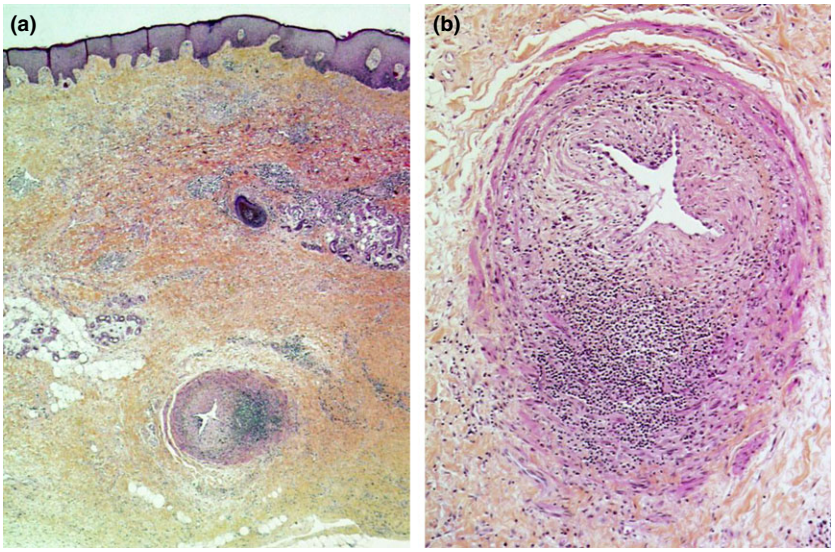

Figure 3 (a) A vessel showing changes of graft vasculopathy (wall thickening, narrowed lumen, lymphocytic cell infiltration) is present in the dermis. (b) higher magnification of panel a.

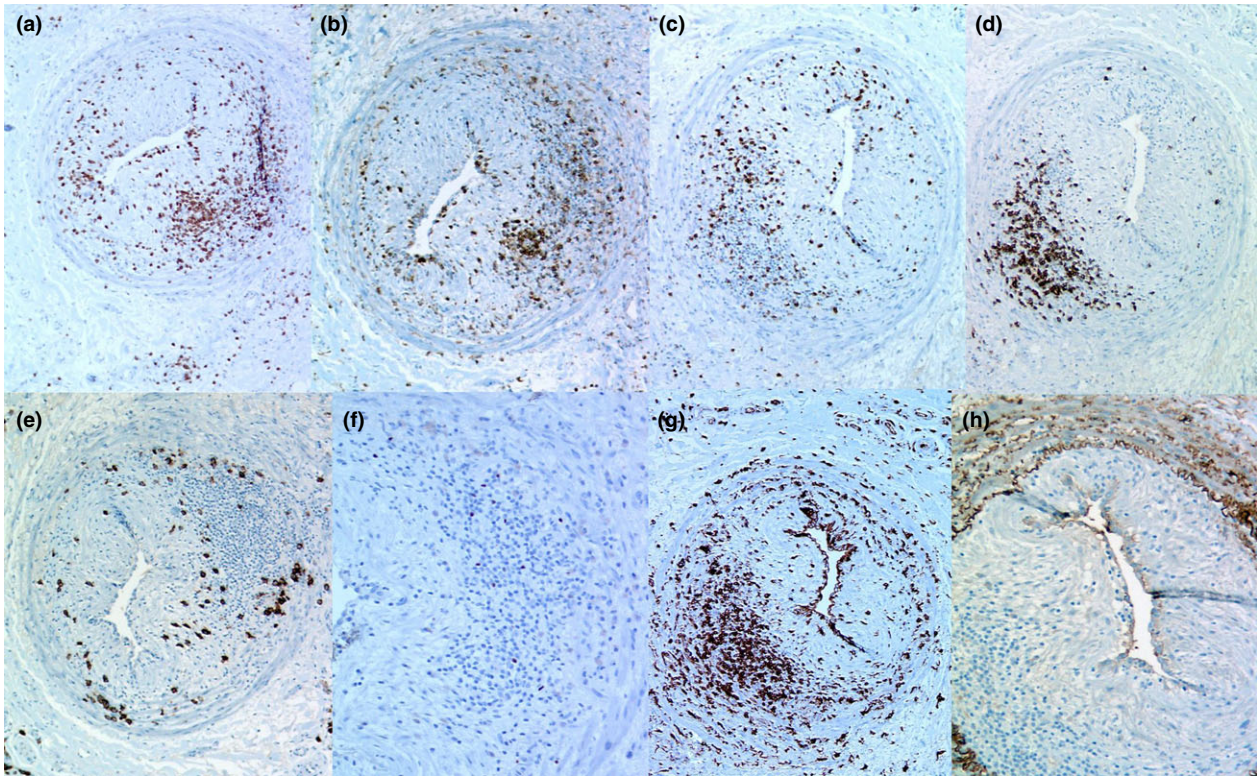

Figure 4 A Skin vessel with graft vasculopathy (shown in Fig. 3a and b) is infiltrated by $CD3^+$ (a), $CD4^+$ (b), $CD8^+$ (c) T cells, $CD20^+$ B cells (d), $CD138^+$ plasma cells (e) and $FoxP3^+$ T-reg cells (f). Infiltrating cells express HLA-DR (g). Endothelial cells express HLA-DR (g) but not C4d (h).

Discussion

Acute rejection often develops in VCA and can usually be reversed upon immunosuppressive treatment increase. Although VCA can undergo severe rejection because of low levels of immunosuppression (that can be due at least partly to patient's nonadherence to the immunosuppressive treatment) [9], GV has been observed only exceptionally in human VCA. Preclinical studies of allotransplantation of hind limbs in rats [10] and face in primates [11] showed that repeated episodes of AR can lead to chronic rejection, manifesting with GV; this scenario likely occurred in the previous patients with GV [6,7] and in the patient presented here, who did not adhere to the immunosuppressive treatment and developed several episodes of AR incompletely treated prior to the development of severe rejection justifying allograft removal.

The immunohistological findings in this patient, disclosing severe changes in a large artery and the skin, and less severe ones in the other tissues, supports the contention that the skin is one of the main targets of rejection in VCA. In our patient, GV affected not only deep vessels, but also medium-sized ones present in the mid-dermis. This observation is important regarding diagnosis of VCA rejection. Recommendations for monitoring human VCA rejection include the use of 4-mm punch biopsies [4,12]. The observation of GV in deep vessels casted some shadow on the adequacy of this procedure, as deep vessels are not included in skin biopsies [7]. Our observation of GV detected in the skin, at a level (mid-dermis) that is included in scalpel or 4-mm punch skin biopsies, suggests that, although vascular imaging studies (e.g., ultrasound biomicroscopy) may be useful to study deep vessels and diagnose GV [7], skin biopsy may also be a reliable method to detect GV in the VCA setting.

The mechanism of GV in VCA remains poorly understood. In solid organ transplantation, chronic vascular damage has been attributed to antibody-mediated rejection (ABMR) [13]. In VCA, the existence of ABMR has not been convincingly shown, either in animals [14] or humans [15], although very recently a case of ABMR was reported in a HHA [16]; however, the pathological changes in that case were characterized by nodular lymphoid aggregates in the skin and did not disclose features of GV. In our patient, DSA were not detected during the first 11 years postgraft, although it was not possible to check his more recent DSA serological status. The usefulness of C4d immunostaining in the diagnosis of VCA rejection is questionable [17], as

dermal capillary C4d deposits are rarely found in VCA [8], and even if present, they are not closely associated with DSA [18]. Our present findings further cast shadow on the diagnostic usefulness of C4d, as such deposits were not found in the vessels most severely affected by GV. Noteworthingly, however, the absence of C4d deposits does not exclude ABMR [13], as C4d-negative rejection admittedly exists in kidney allografts [19]. In our patient, the presence of B cells and plasma cells around and within the wall of vessels with GV is consistent with an involvement of B cells in rejection, although the role of intragraft B cells is ambiguous [20]. The fact that the majority of skin-infiltrating cells of this patient consisted of $CD3^+$/$CD4^+$ T cells, as in AR [3,17] speaks in favor of a predominantly T-cell-mediated mechanism responsible for GV. The finding of T-reg cells within vessels affected by GV suggests that their presence in VCA is not sufficient to prevent rejection. Indeed, we have observed in other patients with VCA that T-reg cells may be present in significant amounts during severe (Banff grade III) rejection (unpublished data).

Whether chronic rejection would occur in human VCA remained until recently unclear. The commonest type of rejection in VCA is AR that develops early postgraft and manifests pathologically with the changes included in the 2007 Banff classification. The significance of changes suggestive of GV in a HHA can be questioned as similar changes may develop after tissue replantation, probably favored or induced by ischemia–reperfusion injury and secondary surgeries [21]. These events may also play a role in HHA; however, our patient presented severe macroscopic skin changes and pathologically also typical findings of rejection (dermal perivascular inflammation), so that, at least in this patient, GV most likely reflects (chronic) allograft rejection. If definitively confirmed by further observations, GV merits to be included in a future update of the Banff classification of VCA rejection, corresponding to grade V (chronic or vascular) rejection. This seems to be more deleterious than AR, as it led to graft loss in all patients who developed this complication [6,7, this case]. In human VCA, GV fortunately remains rare; although it can be expected that it could develop even in patients with adequate immunosuppression, nonadherence of patients to the immunosuppressive treatment obviously favors GV. This underlines the importance of careful selection of patients who will eventually receive a VCA.

Authorship

JK: interpreted the pathological findings of the skin and wrote the paper. GK: interpreted the pathological findings of deep tissues (arteries, muscles, nerves). ML: performed the allograft, followed the patient clinically and removed the allograft after rejection. PP: followed the patient clinically and immunologically.

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