Determining the Histopathology of Hyperacute Rejection

Alan G. Rose

Graft 2001; 4; 14

Determining the Histopathology of Hyperacute Rejection

Alan G. Rose

Terminology

Before discussing what progress has been made in the pig-to-primate model in "determining the histopathology of hyperacute rejection," one should first define what is meant by the term hyperacute rejection (HAR). The term, initially coined by Kissmeyer-Nielsen et al,[1] is applied to the very rapid graft destruction that characteristically occurs within minutes to hours after xenotransplantation. It is arbitrarily defined as developing within 24 hours following transplantation. Subsequent experimental work, which tried to delay or prevent HAR, has rendered this time limitation obsolete. The pathologic process of HAR may be delayed beyond 24 hours and this has led to the introduction of other terms for the same basic process. The latter terms include delayed vascular (hyperacute, humoral, antibody-mediated) rejection.[2] Humoral or antibody-mediated rejection has now become a synonymous term for hyperacute rejection.

The terminology of antibody-mediated rejection has become even more confusing by the use of the unqualified term "vascular rejection". This term[3] has been applied to the following entities:

- hyperacute (humoral, antibody-mediated) rejection;
- delayed vascular (hyperacute, humoral, antibody-mediated) rejection;
- acute vascular (humoral) rejection, also termed the xenograft reaction and microvascular rejection;
- necrotizing arteritis (accelerated rejection);
- endothelialitis of acute rejection, and
- graft vascular disease (chronic rejection).

Terminology would be far less confusing if the term "hyperacute rejection" were to be applied to all forms of antibody-mediated rejection. In order to prevent confusion with the older literature, classical HAR would occur within 24 hours after transplantation and delayed HAR would be the same process encountered later than 24 hours. Consequently, the various forms of rejection that may be encountered in grafts are listed in Table 1.

Histopathology of HAR

The histopathology of HAR has been little studied and there are relatively few substantive publications in the field.[4-7] Most pathologists have little, if any, experience with HAR and this also applies to many highly-skilled subspeciality pathologists, including cardiac pathologists, hepatopathologists, renal pathologists, etc. Standard texts[8] state that preformed antibodies initiate vascular injury in the graft with diffuse hemorrhage, edema, intracapillary fibrin-platelet thrombi, vascular necrosis, and infiltration of neutrophils. The widely-accepted concept of the pathogenesis of HAR is that preformed antibodies destroy the endothelial lining of the graft, leading to secondary hemorrhage, thrombosis and necrosis of the graft. Due to disruption of the capillary microcirculation, it was believed that the antibodies destroyed mainly the capillaries, since they consist only of endothelial cells resting on basement membrane.

Recent morphologic studies have cast doubt on this readily-perpetuated myth.[9] Aziz et al,[9] in a transmission electron microscopy study of HAR guinea pig hearts in rat recipients, noted only increased microvascular permeability and an intact microvascular endothelium devoid of cell injury. Rose and Cooper[10] have shown that microvascular

Alan G. Rose, M.D., F.R.C.Path
University of Minnesota,
Box 76 Mayo Building
420 Delaware St SE,
Minneapolis, Minnesota, USA 55455
Tel.: 612.273.5758
Fax: 612.273.1142
email: rosex031@tc.umn.edu

Table 1 | **Classification of Organ Rejection**

I. Hyperacute Rejection
Classical (onset < 24 hours)
Delayed (onset > 24 hours)
II. Acute Rejection
III. Mixed Delayed Hyperacute And Acute Rejection
IV. Graft Arteriopathy (Chronic Rejection)

Table 2 | **Histologic Grading of Hyperacute Rejection***

Stage 1: Initial (mild)
Normal apart from:
Venular thrombi
Swollen capillary endothelium
Stage 2: Intermediate (moderate)
All of the above plus:
Interstitial edema
Congestion, sludging of capillary erythrocytes
Scanty capillary thrombi
Stage 3: Late (severe)
All of the above plus:
Disruption of capillaries draining into thrombosed venules, resulting in interstitial hemorrhage
Areas of parenchymal necrosis
Thrombi in some arteries

* Based on reference 3.

The venous circulation of the heart has received scant attention by pathologists and in normal histologic and cardiovascular pathology texts.

venular thrombosis is the initial key event in cardiac HAR. This leads to congestion of the subtended venules and capillaries accompanied by interstitial edema and later hemorrhage, which affects the inner (subendocardial) layers of the myocardium. The histopathology suggests that antibody-mediated endothelial damage occurs primarily in the cardiac venules. Venular thrombosis would also explain the transmission electron microscopic observations made by Aziz et al.[9] Cattell and Jamieson,[11] in an ultrastructural study of HAR guinea pig-to-rat xenografts, noted endothelial cell damage, particularly in veins. The present author has observed venular thrombi in HAR affecting renal xenografts also.

Based on this postulated key role for venular thrombosis in the pathogenesis of HAR, a histopathologic grading system of HAR for possible future clinical use has been proposed (Table 2).[3]

Cardiac Veins and Venules

The venous circulation of the heart has received scant attention by pathologists and in normal histologic[12] and cardiovascular pathology texts.[13] The normal appearance of a cardiac vein is not even illustrated in a current detailed text of human histology.[12] Whilst the epicardial veins are easily delineated by their close association with the epicardial coronary arteries, the thin-walled intramyocardial venules are difficult to discern due both to their fine structure (endothelial cells enclosed by collagen fibrils) and their normally collapsed state (negative internal pressure). Intramyocardial veins have a structure more closely allied to capillaries than to veins in other sites in the body. Both types of vessels consist essentially of endothelial cells resting upon a basement membrane, but the intracardiac veins have a thin layer of collagen fibers external to the basement membrane and a much larger diameter than a capillary. Since the veins often have a negative internal pressure, they may appear collapsed and the larger diameter is a potential one. Such venules are more easily seen when distended by thrombus. If the capillaries, which drain into the thrombosed veins, are disrupted by hemodynamic forces following the thrombosis, the residual venules may be mistaken for capillaries unless their greater diameter is noted. Care also has to be taken not to mistake natural

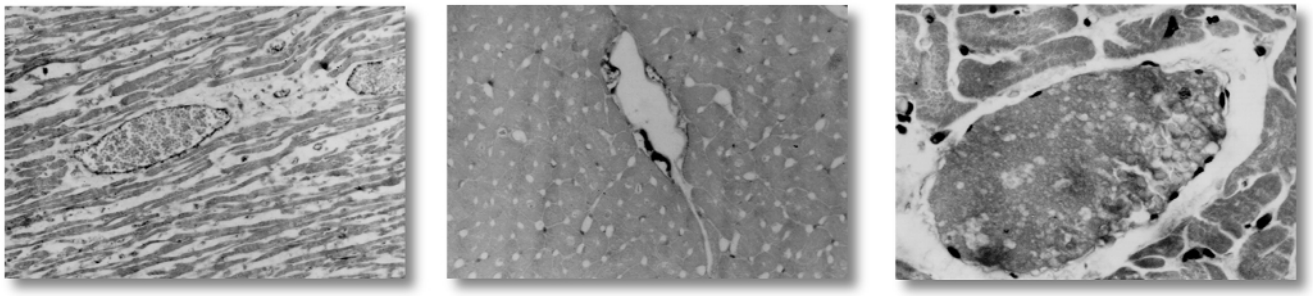

Figure 1. (Left) Control heart showing two interstitial venules filled with erythrocytes and having walls composed mainly of endothelial cells that stain positive for CD31. Note the non-staining of the myocyte nuclei. (Immunoperoxidase stain for CD31antigen x 60). (Center) Formalin coronary perfusion-fixed heart shows an intermyocyte venule composed of CD31positively-stained endothelial cells surrounded by minimal connective tissue (x 180). (Right) Hyperacutely rejecting cardiac xenograft shows a small venule distended by thrombus. A thin band of endothelial cells clearly encompasses the thrombus (H & E, x200).

spaces between fascicles of myocytes, which are devoid of an endothelial cell lining, for venular structures. Positive staining with CD31 is useful in distinguishing true venules from such "myotubules."

Delay of HAR by immunosuppression or other means may allow time for organization of some venular thrombi. Biopsies from organs thought to exhibit HAR should be interpreted in the light of the site from which the biopsy comes (e.g., epicardial or endomyocardial in the case of the heart), as well as the details of the vascular arterial supply and venous drainage (e.g., dual circulation in the liver). Thus, cardiac HAR will show the classical features of capillary disruption and interstitial hemorrhage in the inner half of the myocardium, whilst a sub-epicardial biopsy may show only interstitial edema. In hepatic HAR a reticulin stain may be useful in identifying thrombi within centrilobular venules. Caution should be exercised in interpreting the significance of neutrophils within a graft showing HAR since capillary hemorrhage will introduce both erythrocytes and white blood cells into the interstitium in the same relative proportions as in the circulation.

. . . cardiac HAR will show the classical features of capillary disruption and interstitial hemorrhage in the inner half of the myocardium, whilst a sub-epicardial biopsy may show only interstitial edema.

Conclusion

The terminology of HAR has become outmoded due to advances that have been made in delaying its onset. It is recommended that the term hyperacute rejection should be applied to antibody-mediated rejection, with classical hyperacute rejection occurring within 24 hours and with delayed hyperacute rejection being the same process but being encountered after 24 hours (Table 1). Recognition of the key role that venous thrombosis plays in the pathogenesis of HAR will allow the microscopist to intelligently interpret biopsies from various portions of a transplanted organ according to the obstructed venous drainage of the organ. Once xenografting becomes feasible, it will be possible to apply a histopathologic grading system to HAR in clinical practice.

References

1. Kissmeyer-Nielsen F, Olsen S, Peterson VP et al. Hyperacute rejection of kidney allografts associated with preexisting humoral antibodies against donor cells. **Lancet 1966; 2:662-665**.
2. Bach FH, Robson SC, Ferran C et al. Endothelial cell activation and thromboregulation during xenograft rejection. **Immunol Rev 1994; 141:5-30**.
3. Rose AG, Cooper DKC. A histopathologic grading system of hyperacute (humoral, antibody-mediated) cardiac xenograft and allograft rejection. **J Heart Lung Transplant 1996; 15:804-817**.
4. Rose AG. Pathology of xenograft rejection. In: The Transplantation and Replacement of Thoracic Organs (first edition). Cooper DKC, Novitzky D, eds. **London: Kluwer Academic, 1990:479-483**.
5. Rose AG, Cooper DKC, Human P et al. Histopathology of hyperacute rejection of the heart—experimental and clinical observations in allografts and xenografts. **J Heart Lung Transplant 1991; 10:223-234**.
6. Rose AG. Histopathology of cardiac xeno-graft rejection. In: Xenotransplantation (first edition). Cooper DKC, Kemp E, Reemtsma K, White DJG, eds. **Heidelberg: Springer, 1991:231-242**.
7. Forbes RDC, Guttmann RD. Histo-pathology and mechanisms of rejection in xenotransplantation. In: Xenograft 25. Hardy MA (editor). **New York: Elsevier, 1989:133-147**.
8. Jennings RB, Steenbergen C Jr. The heart. In: Pathology (Third edition). Rubin E, Farber JL (editors). **New York: Lippincott-Raven, 1999: 533-588**.
9. Aziz S, Suzuki K, Thorning D. Mechanism of discordant cardiac xenograft rejection—an alternative view based on ultrastructural observations. In: Xenotransplantation (second edition). Cooper DKC, Kemp E, Platt JL, White DJG (editors). **Heidelberg: Springer, 1997: 273-286**.
10. Rose AG, Cooper DKC. Venular thrombosis is the key event in the pathogenesis of antibody-mediated cardiac rejection. **Xenotransplantation 2000; 7:31-41**.
11. Cattell V, Jamieson SW. Hyperacute rejection of guinea-pig to rat cardiac xenografts. I. Morphology. **J Pathol 1975; 115:183**.
12. Billingham ME. Normal heart. In: Histology for Pathologists. Sternberg SS, ed. **New York: Raven Press. 1992:215-231**.
13. Ferrans VJ, Thiedeman KU. Ultrastructure of the normal heart. In: Cardiovascular Pathology. Silver MD, ed. **New York; Churchill Livingstone. 1983:31-86**.