Unusual Apoptosis in Experimental Cardiac Rejection

To the Editor-in-Chief:

Being interested in the histopathology of cardiac rejection,1 I was pleased to read the constructive article by Miura and co-workers2 about the transplantation of hearts from A/J (H-2a) mice to major histocompatibility complex mismatched recipients divided further into two groups: wild-type (WT) C57Bl (H-2b) and interferon (IFN)-γ deficient C57Bl/Ifn-γ-/- mice. The allografts were rejected in about 8.5 days in WT recipients by a severe cellular acute rejection mediated by CD4+ T cells and in about 6 days in IFN-γ deficient hosts by a rejection manifesting intense infiltration by neutrophils. The analysis of Figure 2 of Miura et al’s2 article, visualizing basic histopathology of the rejecting cardiac allografts about 1 day before the end of rejection, is the subject of this letter.

In substance, I agree with the authors as far as Figure 2, a and c, are concerned. There are still some well-preserved cardiomyocyte nuclei in Figure 2c. I mention this fact because absolute magnification numbers accompanying published microphotographs are often imprecise due to printing procedures. I will compare, therefore, the approximate diameter of cardiomyocyte nuclei (4 μm) in Figure 2c (×200) with one structure in Figure 2d (×200) to determine its dimensions.

I also agree with the authors regarding the neutrophil infiltration in Figure 2, b and d. However, I do not see “diffuse intense intragraft thrombosis” in Figure 2, b and d. It is quite possible that small microthromboses exist in the heart in question, but I have not noticed any identifi-

able thrombus in this figure. What factual information supports the “thrombosis” statement?

I have been struck most by a formation of myocardial defects which are well visible in Figure 2b. One of them is seen in detail in Figure 2d. These defects may be mistaken for vessels at first sight. However, they manifest the features which are incompatible with vascular origin: they do not possess vessel walls; they often fuse gradually with the surrounding myocardium (Figure 2d, upper right quarter); they contain fragments of cardiomyocyte cytoplasm (Figure 2d, lower left center, at the “7 o’clock” position); and they comprise cells with nuclei similar to the nuclei of cardiomyocytes and surrounded by a narrow rim of cytoplasm with cardiomyocyte tinctorial properties (Figure 2d). Some of these nuclei are practically “naked.”

What pathological process has created such myocardial defects within five days after transplantation? Is it the necrosis mediated by neutrophils and mentioned by the authors?3 Myocardial necrosis is phagocytized by macrophages. This is a process lasting days, weeks, and months which is followed immediately by healing reaction and scarring. Consequently, necrosis would not have formed myocardial defects filled with interstitial fluid (Figure 2, b and d), and one must look for another explanation for their appearance.

To do so, let’s pay attention to the “severe disseminated hemorrhagic necrosis,” which is the third important pathological process mentioned by the authors in Figure 2, b and d.2 In cardiac pathology, the term “hemorrhagic necrosis” is used to designate necrotic myocardium with blood extravasated into the interstitial space. It is often described in hemorrhagic infarcts, hyperacute rejection, and other pathological processes. Its concept suffers, however, from numerous shortcomings. For example, interstitial spaces between cardiomyocytes are extremely narrow (from 0.2 μm to a few μm) and blood pressure is not high enough to dislodge cardiomyocytes from their original position.4 It is difficult, therefore, to account for large accumulations in red cells in the narrow interstitial spaces. Most often, alleged extravasated blood contains only erythrocytes and lacks an adequate amount of fibrin. Furthermore, it is a process which is accompanied by an unaccounted loss of cardiomyocytes.5 To explain these contradictions, one current theory proposes that alleged red cells present in “hemorrhagic necrosis” are mostly cardiomyocyte apoptotic bodies similar to erythrocytes.3–6 Only later, when the interstitium is no longer supported by intact cardiomyocytes, may vessels become injured and give rise to genuine hemorrhage.6

If this new hypothesis is correct, where are the cardiomyocyte apoptotic bodies in Figure 2, b and d? Unquestionably, some were already phagocytized by macrophages, many were transported away by lymphatic outflow,3 and most are still in the tissue being considered to be red cells by the authors.2 One may see them best in “naked” red cells by the authors.2 One may see them best by Miura and co-workers2 about the transplantation of hearts from A/J (H-2a) mice to major histocompatibility complex mismatched recipients divided further into two groups: wild-type (WT) C57Bl (H-2b) and interferon (IFN)-γ deficient C57Bl/Ifn-γ-/- mice. The allografts were rejected in about 8.5 days in WT recipients by a severe cellular acute rejection mediated by CD4+ T cells and in about 6 days in IFN-γ deficient hosts by a rejection manifesting intense infiltration by neutrophils. The analysis of Figure 2 of Miura et al’s2 article, visualizing basic histopathology of the rejecting cardiac allografts about 1 day before the end of rejection, is the subject of this letter.

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approximate diameter of cardiomyocyte nuclei in Figure 2c (4 μm) while red cells have a diameter of approximately 7.2 μm. In the myocardium surrounding the defects, both individual apoptotic bodies and their conglomerates may be seen. It is difficult to reconcile large dimensions of the conglomerates with narrow interstitial spaces. In reality, the conglomerates enter into intimate contact with cardiomyocyte cytoplasm and are sometimes entirely surrounded by it. All these features indicate that the alleged red cells are cardiomyocyte apoptotic bodies. Consequently, the main mechanism of cardiomyocyte apoptotic bodies has been described in the humoral rejection of human cardiac allografts.1

Does the experimental system visualized in Figure 2, b and d, permit cardiomyocyte apoptosis to take place? The answer is yes. Cardiomyocytes possess death receptors (Fas, tumor necrosis factor receptor, etc) and neutrophils have Fas ligand, tumor necrosis factor-α, etc.6 In certain conditions, cardiomyocyte receptors and neutrophil ligands enter into contact resulting in cardiomyocyte apoptosis. Why hasn’t this striking phenomenon been described sooner? Firstly, it may have been overlooked and secondly, it may take place only in special situations such as a deficiency of interferon-γ in transplantation recipients.

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References

3. Beranek JT: Quick disposal of dead cardiomyocytes: an ultimate consequence of histocompatibility complex-disparate cardiac allografts in IFN-γ deficient recipients. As mentioned by Dr. Beranek, the presence of a source of interferon-γ in the absence of an IFN-γ-deficient recipient is an important point of this report. That is, when we initiated these studies we had expected to observe unregulated expression of neutrophil chemoattractants (eg, KC/Groα and MIP-2) in the allografts retrieved from the IFN-γ-deficient recipients. As pointed out by Dr. Beranek in a recent letter to The American Journal of Pathology,4 we hypothesized that neutrophils might play a critical role in this histopathology.

Authors’ Reply:

We appreciate the interest and many positive comments made by Dr. Beranek concerning our recent report investigating the rejection of MHC-mismatched cardiac allografts in the absence of IFN-γ.1 The goal of this study was to investigate mechanisms mediating the rapid rejection of organ allografts in the absence of IFN-γ. This rapid rejection has been observed in murine models of renal, heart, and more recently liver allografts where either the recipients are unable to produce IFN-γ or the grafts are from IFN-γR−/− donors and are therefore unable to respond to IFN-γ produced by graft-infiltrating T cells.2–6 A finding that is always observed during the rapid rejection of renal, cardiac, and liver allografts in these models is severe graft tissue necrosis and hemorrhage that accompanies the rejection. Rejection of the control allografts in the presence of IFN-γ is delayed in comparison and characterized by increasing mononuclear cell infiltration that eventually results in graft failure. Based on the necrosis observed in the absence of IFN-γ, we hypothesized that neutrophils might play a critical role in this histopathology.

Dr. Beranek has astutely pointed out fine details of the histopathology in the allografts from IFN-γ−/− recipients depicted in Figure 2 of our report. Several comments are in order regarding his evaluation of the sample sections shown. First, we were of the opinion from the first viewing of the structure shown in the lower left-hand corner of Figure 2d that it is not a vessel for many of the reasons stated by Dr. Beranek. Second, we did observe many small thromboses in vessels throughout the graft although these were not shown in the figure. Third, Dr. Beranek makes a good point with regard to the small bodies in the figure panel that may or may not be erythrocytes or apoptotic bodies. We have not investigated the presence or temporal aspects of myocardial apoptosis in this model. However, graft tissue hemorrhage has been observed in other solid organ allografts retrieved from IFN-γ deficient recipients with similar histopathological features shown in Figure 2, b and d.2–6 It will be of some importance to distinguish these features in the rejection of these heart allografts but as Dr. Beranek points out this may represent a very specialized case of tissue pathology. A potential solution might be the use of tissue factor staining to distinguish erythrocytes from myocardial apoptotic bodies as pointed out by Dr. Beranek in a recent letter to The American Journal of Pathology.4 Fourth, Dr. Beranek has asked what pathological process generates the myocardial defects shown in these grafts after only 5 days. The data of the report are strongly supportive of a neutrophil-mediated mechanism that occurs as rapidly as shown. In our view, this histopathology looks like neutrophil-mediated necrosis and may include neutrophil-mediated apoptosis of cardiomyocytes. It is important to state again that similar patterns of histopathology are observed in other organ allograft models in the absence of IFN-γ.

Finally, Dr. Beranek concludes with what we feel is the most important point of this report. That is that IFN-γ is an important regulator of early innate immune attack on the allograft. In the absence of a source of IFN-γ the allografts are intensely infiltrated with neutrophils and quickly exhibit the tissue necrosis depicted in Figure 2 of the report. With this in mind one should be asking what regulatory aspects of IFN-γ protect the allograft from this pathology. When we initiated these studies we had expected to observe unregulated expression of neutrophil chemoattractants (eg, KC/Groα and MIP-2) in the allografts retrieved from the IFN-γ-deficient recipients. As
shown in Figure 5 of the report this is not the case. These results raise an important and unanswered question regarding the IFN-γ-dependent mechanism(s) that restrict the temporal infiltration of neutrophils into the allograft to mediate this extreme histopathology. This continues to be a focus of our studies to fully understand and minimize this attack.

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References

Explaining Decreased Nitric Oxide Production in Psoriatic Lesions: Arginase 1 Overexpression versus Calcitonin Gene-Related Peptide

To the Editor-in-Chief:

I read with great interest the paper written by Bvuch-Gerharz et al., published in the January 2003 issue of The American Journal of Pathology. In this paper, the authors have explained the reason for the low NO concentration in the psoriatic plaques, in the face of high expression of inducible NO synthase (iNOS) mRNA and protein, by showing that arginase 1, which substantially regulates iNOS activity by competing for the common substrate L-arginine, is highly overexpressed in the psoriatic epidermis.

This is a feasible explanation, but not the only one. As a complement to the explanation for the low NO concentration in psoriatic plaques, I would like to mention the effects of calcitonin gene-related peptide (CGRP) on nitric oxide generation. The pathogenesis of psoriatic plaque lesions is closely related to the overexpression of CGRP and it has been shown that CGRP-containing nerve fibers are more dense in the psoriatic epidermis. Taylor and co-workers have shown that CGRP suppresses the production of NO most probably through inhibition of iNOS enzymatic activity.

Therefore, it could be concluded that in addition to the overexpression of arginase 1, overexpression of CGRP in the psoriatic lesions could decrease the production of NO, thereby preventing the NO concentration to reach the keratinocytostatic levels.

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References

Author’s Reply:

In the letter by M. R. Namazi, the interesting idea is put forward that in addition to our demonstration of arginase 1 overexpression, calcitonin gene-related peptide (CGRP) might contribute to depressing the iNOS activity in psoriatic plaques. His suggestion is based on two observations: a known overexpression of CGRP in psoriasis and a previous publication on the suppressive activity of ocular aqueous humor on NO synthesis being due to the presence of CGRP.

We were well aware of these findings, however, as relates to a CGRP-mediated inhibition of iNOS activity, there are controversial data in the literature. In a series of carefully controlled experiments it has also been shown that CGRP actually enhances iNOS expression and activity with doses of CGRP that were both lower and higher as in the first study. Moreover, in ocular aqueous humor the presence of several other factors with known and confirmed depressive action on iNOS activity had been characterized subsequently by the same group. It thus appears that depending on the presence of additional factors, CGRP may do both, either further enhance or additionally depress NO formation. And in this respect there is no way to currently estimate whether a hypothetically increased presence of this peptide in the epidermal layer might contribute to suppression of NO formation.