Endothelial Cells and Radiation Gastrointestinal Syndrome

Paris et al. have quantified extensive apoptosis in endothelial cells of jejunal microvasculature (1). This raises questions about the pathobiology of tissue injury from irradiation, but their interpretation that these changes are the basis for postradiation denudation of the jejunal mucosa is not supported by relevant data.

If death of irradiated jejunal crypt cells were due principally to apoptosis of the capillary endothelial cells and consequent blockage of capillary blood flow, the mechanism would be hypoxic or ischemic death. In this scenario of severe jejunal hypoxia, the slope of the crypt cell survival curve (log cell survival fraction versus dose) would be steep for aerobic cells versus dose (2) would be steep for aerobic cells because principal to apoptosis. Cells in the mitotic zone have been shown to be more sensitive to apoptosis than clonogenic crypt cells, and the kinetics of apoptosis and recovery are different. The authors presented no evidence that the endothelial cells were regulating apoptosis or, when damaged, initiating injury in the epithelial clonogenic (regenerative) cell population.

In vivo colony techniques (2), rather than apoptosis levels, should be used to assess the contribution of the epithelium to GI syndrome severity. There are a few apoptosis-susceptible cells in the crypt stem cell zone considered to act as a protective mechanism for the organism against mutations that, after low doses of radiation, might have carcinogenic potential (3, 4). After high cytotoxic doses, epithelial regeneration proceeds from a few surviving cells out of a larger clonogenic population (5–7). The relationship between these two populations is not yet resolved, but clearly, it is the latter population that should be compared to the endothelial response when analyzing GI syndrome.

Radioprotection by basic fibroblast growth factor (bFGF) of LD50/6 by 10%, as well as the apoptosis-susceptible crypt cells and the epithelial clonogens, has been reported (8, 9). Although there have also been several reports of bFGF reducing endothelial apoptosis in culture, evidence for its efficacy in vivo—such as in the lung, where endothelial cells may play a role in radiation-induced pneumonitis—is conflicting (10, 11). Hence, although the work of Paris et al. showed that changes can be made in radiation-induced apoptosis in gut endothelial cells, and concomitantly in the GI syndrome, the relative contribution of endothelial, as opposed to epithelial, injury in the syndrome remains to be established.

References
2. H. R. Withers et al., Cancer 34, 59 (1974).

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Response: This discourse focuses on the role of endothelial apoptosis in radiation-induced GI injury. Hendry et al. argue that the relative contribution of endothelial apoptosis, as opposed to epithelial injury, remains to be established. We agree with these investigators that dysfunction of clonogenic crypt epithelial cells, rather than lethality, constitutes a critical element in the pathogenesis of GI damage. The early mitotic arrest occurring in these cells, coupled with their continued migration toward the extremism zone at the villus apex, results in cellular depletion of the crypt while maintaining the villus mass (1). Shrinkage of the villus commences after crypt depletion is completed, leading to involution of the mucosa and the GI syndrome (1).

Our data on the effects of the as34ase--/ mutation and bFGF treatment provided compelling evidence for linkage between endothelial and epithelial damage. The fact that crypt epithelial cells lacked bFGF receptors either before or after irradiation (2) precludes direct effects of bFGF on the level of radiation-induced dysfunction or the mitotic recovery of these cells. These observations order microvascular endothelial apoptosis upstream of crypt epithelial death in the evolution of the GI syndrome. The precise mechanism of the endothelial-epithelial linkage remains unknown, although ischemia due to microcirculatory impairment likely plays a role in this process.

Hendry et al. also comment that there is little evidence that endothelial cells are more sensitive to apoptosis than clonogenic crypt epithelial cells. However, our data (2) showed that endothelial apoptosis plateaus at 4 hours after 15 Gray (Gy), but that crypt epithelial cells displayed no discernible apoptosis, except at position 4 to 5, which is not associated with the GI syndrome (1). Further, recent dose-escalation experiments have shown that at 18 Gy, intensive epithelial apoptosis develops at positions 6 to 10 from the crypt base (3). This response is subject to different biochemical regulation than the en-
dothelial response, and is amenable to genetic and pharmacological manipulations different from those controlling endothelial apoptosis (3). These observations indicate that the epithelial clonogens of the crypt are indeed more resistant to lethal effects of radiation than the GI microvascular endothelium. The data also suggest a hierarchy in the sensitivity of critical tissue elements of the GI tract to different modes of radiation-induced cell death, activated in an orderly fashion as the dose increases (3).

Suit and Withers argue that the differential effects of radiation on the crypt and villus are inconsistent with our notion of microcirculatory impairment and ischemia in the pathogenesis of the GI syndrome. Concurrent damage to both crypts and villae would have rather been expected if blood flow were blocked. However, our results did not indicate an infarctlike, acute tissue necrosis. Instead, our data showed that even at doses that induce the GI syndrome, the severity of endothelial apoptosis was heterogeneous, with some regions exhibiting extensive damage and others showing mild or no apoptotic damage at all. In many instances, extensive endothelial apoptosis was observed at the upper half of the villus and none in the regions adjacent to the crypt, or vice versa. The paper’s figures clearly demonstrated these points (2). Hence, it appears that the microcirculatory damage leads to focal regions of ischemia of differing intensities, rather than to global tissue hypoxia.

Suit and Withers further suggest that if our model of microvascular dysfunction is correct, it would predict that curves of crypt regeneration versus dose would have progressively more shallow slopes as the number of radiation fractions increases. In fact, published data demonstrated a progressive flattening of jejunal crypt regeneration curves as the fraction number increased from 1 to 5, 10, and 20 fractions [figure 3 in (4)], but detailed statistical analysis of these curves was not provided. We have digitized the data from the original publication and statistically analyzed the slopes based on pairwise sets of points within a fraction. This analysis confirmed that the slopes were indeed different (5).

Suit and Withers also comment that data from a split radiation dose experiment published by Withers (6), in which two radiation doses were delivered at interfractional intervals of 1 to 48 hours, are inconsistent with our model. They argue that hypoxia produced by microvascular damage from the first radiation dose should have induced resistance to the second exposure, but this pattern was not observed. It should, however, be noted that in this particular experiment, the lead dose was only 660 cGy, a dose that, according to our data, produces minimal if any endothelial apoptosis and microvascular dysfunction (2). Hence, hypoxia-mediated radiation resistance to the second dose would not have been anticipated.

Finally, it should be emphasized that the hypothesis of a direct lethal effect of radiation on GI stem cells has never been proven experimentally. It is impossible to assess parameters of damage and repair, survival or death in a cell that has no markers and whose existence can only be inferred. The hypothesis on radiation-induced clonogenic stem cell death was derived by analogy from mathematical and biophysical models developed to fit survival data and explain patterns of mammalian cell responses to irradiation in tissue cultures. However, it should not be forgotten that the intestine is a complex organ, comprising many different interacting cell types and structures, with distinct response patterns to radiation. We believe that the overall response to radiation reflects a complex interplay of these cells, which cannot be interpreted at present by hypothetical models, but rather must be elucidated using molecular and genetic reagents in vivo.

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