Reply

I agree with Dr. Brown (1) that cell cycle checkpoint defects do not play a major role in radiosensitivity of AT cells and that irradiated AT cells have an increased residual level of chromosome breaks when compared to that of normal cells (2–5). Our disagreement lies in assessing the relative contributions of residual chromosome breaks *versus* apoptosis to the radiosensitivity of AT cells.

There is a strong inverse correlation between the number of residual chromosome fragments and the probability of reproductive survival for irradiated human cells, whether normal or radiosensitive. However, it should be borne in mind that correlation does not establish causality. In addition, for radiosensitive human cells, the observed correlation between an increased number of residual chromosome fragments and decreased survival is not absolute. *E.g.*, Badie *et al.* (6) found that when compared to the AT fibroblast line AT2, 180BR fibroblasts had significantly higher levels of residual chromosome damage 24 h after X-irradiation, yet they were not as sensitive as AT2 cells to radiation-induced killing.

Although increased levels of residual chromosome damage may contribute to the sensitivity of AT cells to the killing effects of ionizing radiation, they do not quantitatively account for all of the increased radiosensitivity of AT cells. Although the published data are few, they suggest that for low to moderate amounts of radiation, fewer irradiated AT cells survive a given number of residual chromosome breaks than normal cells. *E.g.*, Sasai *et al.* (5) found that for irradiated normal human fibroblasts (AG 1522), ~0.76 residual chromosome 4 breaks/cell were associated with 0.9% survival, whereas for irradiated AT fibroblasts (AT 2052), ~0.67 residual chromosome 4 breaks/cell were associated with 0.1% survival (Fig. 4 from Ref. 5). Work by Pandita and Hittelman (3) may provide additional support. They examined normal and AT lymphoblasts for initial chromosome breaks present immediately after irradiation and residual chromosome breaks present 90 min after irradiation (3). They found that both normal and AT cells removed the majority of induced chromosome breaks in the first 90 min postirradiation. However, for a given number of either initial or residual chromosome breaks, AT cells demonstrate consistently lower survival (3). The sensitivity of AT lymphoblasts was particularly pronounced for cells irradiated in G_1 (Fig. 1). Pandita and Hittelman concluded that although higher levels of chromosome damage contribute to the radiosensitivity of AT cells, "other differences in AT cells must also contribute to their sensitivity phenotype" (3).

Observations as to when and how irradiated AT cells die also argue against the hypothesis that the simple presence of an increased amount of residual chromosome damage caused by a "defect in the rejoining of chromosome breaks" completely accounts for AT radiosensitivity. As discussed in my review (7), most fatally irradiated AT cells irreversibly arrest before their first postirradiation mitosis rather than die after undergoing several mitoses (8–10). If the residual chromosome damage caused by a defect in chromosome rejoining is solely responsible for the death of these arrested cells, then this residual damage apparently acts via a novel mechanism not usually seen in normal cells.

One such novel mechanism is the increased level of radiation-induced apoptosis seen in irradiated AT cells. This excess apoptosis is not restricted to SV40-transformed AT fibroblasts but has also been seen in primary fibroblasts, lymphoblasts, and T lymphocytes

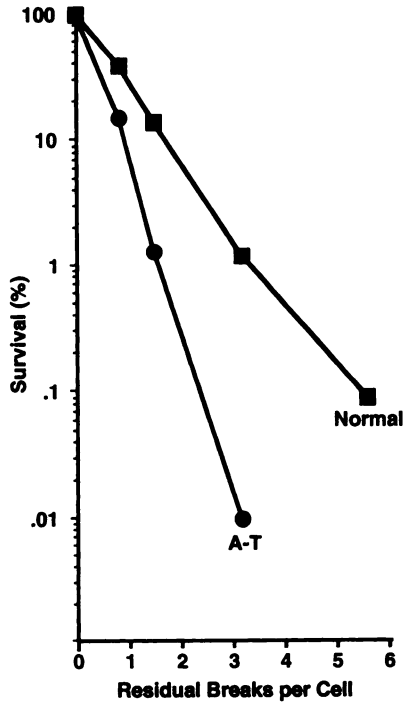

Fig. 1. The relationship between survival (measured by colony-forming ability) and residual chromosome damage in G_1 lymphoblasts (measured at 90 min postirradiation by premature chromosome condensation). Data replotted from Fig. 7, Pandita and Hittelman (3).

(11).[2, 3] Even when using radiation doses that give equal reproductive survival, more irradiated AT cells undergo apoptosis 72–96 h after irradiation than controls (11),[2] indicating that apoptosis is a favored mode of death for lethally irradiated AT cells. When radiation-induced apoptosis is blocked in SV40-transformed AT fibroblasts by inactivation of p53, the AT cells acquire near-normal radioresistance (11),[2] suggesting that p53-mediated apoptosis plays a major but not exclusive role in the radiosensitivity of these cells.

Taken together, studies of residual chromosome damage and apoptosis in irradiated AT cells suggest that higher levels of residual chromosome damage and an increased tendency to undergo apoptosis *together* account for most if not all of the sensitivity of AT cells to the killing effects of ionizing radiation.

Increased residual chromosome damage and excess apoptosis are not mutually exclusive explanations of the radiosensitivity of AT cells, because one hypothesis focuses on the nature of the lethal damage, and the other focuses on the mode of death. In fact, the two may be linked in AT cells. We do not yet know what triggers excess apoptosis in irradiated AT cells. However, in line with Dr. Brown's comment that certain cells may undergo apoptosis after sensing mis-repaired chromosomes, I would suggest that it is possible that in the absence of functional ATM protein, chromosome fragments in AT cells trigger apoptosis, either because the cell senses an unusual structure at the broken end of the chromosome or as a result of an inappropriate cellular response to ordinary chromosome breaks.

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[2] M. S. Meyn, L. Strasfeld, and C. Allen. p53-mediated apoptosis is a major cause of radiosensitivity in ataxia-telangiectasia, submitted for publication.
[3] D. L. McElligott, personal communication.

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