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STUDIES ON THE PROLIFERATIVE CAPACITY OF NORMAL MER-1 CELLS AND SYNGENEIC R-1,M RHABDOMYOSARCOMA CELLS BEFORE AND AFTER IRRADIATION AT DIFFERENT TIME INTERVALS OF GROWTH IN VITRO IN SINGLE AND IN MIXED CULTURES

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One of the characteristic manifestations of malignant tumour growth is the local destruction of the normal tissue by invading tumour cells. However, some types of normal tissue cells are, conversely, capable of responding to the presence of tumour cells by directly or indirectly killing these. During local tumour progression, the numbers of normal and tumour cells which will be mutually killed as a result of cell to cell interactions will depend on the number of normal and tumour cells which are in close contact. After treatment with X-rays, the relative numbers of clonogenic normal and tumour cells which are in close contact may especially change if the two cell types show differences in their responsiveness to radiation. Consequently, the quantity of clonogenic cells of either type which will be killed after therapy as a result of mutual cell interactions may change concomitantly. To study the consequences of interactions between normal and tumour cells with respect to their proliferative capacity before and after irradiation, in vitro experiments were performed as a first approximation. For this purpose, R-1,M tumour cells of the transplantable rhabdomyosarcoma and syngeneic normal MER-1 cells derived from the rat mesentery were grown either in single or in mixed cultures. The rat mesentery cells show a phagocytic capacity when cultured in vitro.

In initial experiments, the time course variations in the number of clonogenic cells were analyzed for each of the two cell types. Cells of either type were seeded into each of a series of culture flasks and subsequently incubated for up to a maximum of 14 days. At 2 to 3 day intervals, the cells from two flasks were harvested, counted and assayed for the fraction of clonogenic cells by replicate in vitro plating of known numbers of cells. Two days after the seeding of 2×10^4 cells per flask, i.e., at a density of 5×10^2 cells/cm² per flask, they started to increase exponentially in number and this continued until plateau phases were reached in the R-1, M and MER-1 cultures at cell densities of 5×10^5 cells/cm², respectively. The fractions of clonogenic cells in the R-1,M cultures varied with time after seeding (at day $t = 0$) from 40 per cent (at day $t = 2$) to a maximum of 85 per cent (at day $t = 6$) and subsequently decreased to 18 per cent (at day $t = 14$). Values for the MER-1, cultures determined for corresponding time intervals were 20, 50 and 18 per cent, respectively.

Values for the same parameters as described above were determined for the two types of cells grown in mixed cultures prepared by seeding 2×10^4 R-1,M cells plus 2×10^4 MER-1 cells into individual culture flasks. For practical reasons, the data on the clonogenic capacity were expressed as the ratio of the number of MER-1 and R-1,M colonies observed per 100 cells used per assay. The results showed that the ratios vary from 0.3 at day $t = 4$ to 0.08 at day $t = 8$ and then increase again to 0.2 at days $t = 12$ and 14. The ratios determined from these data for time intervals of up to days $t = 4$ to 6 are significantly larger than those calculated for corresponding time intervals on the basis of data on fractions of clonogenic cells obtained for single cultures of either type of cell.

It may be concluded from these results that the total number of clonogenic tumour cells are temporarily reduced or, alternatively, the total number of

clonogenic normal cells is increased relative to those present in single cultures of either type of cell.

Finally, radiation experiments were performed in order to determine the radiosensitivity of MER-1 and R-1,M cells at different time intervals of growth in single or mixed cultures. Therefore, monodispersed cell suspensions were prepared at days $t = 0, 4, 8, 12$ and 14 after initiation of the cultures as described above. Appropriate numbers of cells were then plated in plastic culture flasks and subsequently submitted to irradiation with $0, 1, 3, \text{etc.}$, up to 11 Gy of 300 kV X-rays . From the numbers of colonies counted after 8 days of culturing, the fraction of surviving cells was calculated for each dose of irradiation and these data were used to construct dose survival curves from which values for D_0 and D_q were derived.

The results obtained in these experiments demonstrated that the radiosensitivity of the MER-1 cells grown for 4 to 14 days in the mixed cultures had increased as compared to that determined for MER-1 cells grown in single cultures. This increase in radiosensitivity is mainly due to a decrease in the value of D_0 ; i.e., from a value of 2.7 Gy measured for MER-1 cells at days $t = 0$ in single cultures to $D_0 = 0.3$ to 0.4 Gy at day $t = 14$ in mixed cultures. In contrast, the R-1,M cells showed only small changes in values of D_0 .

Further experiments are required to investigate the mechanism responsible for inducing these changes in radiosensitivity in the normal cells.

DISCUSSION

Freshney : Do tumour cells produce growth factors ?

Hermens : It may be possible but when we use the medium in which the tumour cells did grow and add this to the normal cells, we didn't find a change in growth or growth rate.

Freshney : But the factor might be internalized by the cells ?

Hermens : At the other hand the kinetic data do not speak in favour of your concept.

Mareel : Can you count in mixed cultures the individual cells ?

Hermens : We count the number of cells in the individual cultures of tumour and normal cells, in the mixed culture we count the total number and so we know the ratio no cells - tumour cells.

And so you can compare the theoretical ratio and the actual ratio for you know that each colony is derived from a single cell. Cytological distinction between the two cell types is not feasible.

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