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AIRMAIL

Dear George:

Ever since your excellent talk at Harvard on immune surveillance, I have been thinking about alternative ways to explain the phenomenon of "sneaking through".

As I understand it, we are talking about the situation where moderate cell doses of an antigenic, chemically-induced tumor are rejected by the syngeneic host, while very large cell innoculi continue to grow into large tumors and very small injections also grow well.

Let me try this idea on you. Assume that when tumor cells are injected into an animal, there is "antigen spillage" during the few days of avascular phase. This is not the case with spontaneously arising tumors. Therefore, if the cell innoculum is very large, tumor antigen "spilling out" as an artifact of the injection, might continue to circulate in the bloodstream until the tumor was vascularized. At the moment of vascularization, there would be continuous entry of new antigen into the bloodstream. The avascular period following injection is rarely more than 4-5 days. The very large innoculum would be associated with levels of circulating antigen from the first hour after inoculation until the tumor was very large and vascularized, thus preventing immune reaction.

With the medium-size cell innoculum, it is possible that antigen spillage might persist for only 1-2 days. Antigen might then disappear from the circulation for 4-5 days until the tumor was vascularized. By this time, however, the recently vascularized tumor would be subject to immune attack due to the brief antigenic exposure after injection.

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With a very small cell inoculum, let us assume that there is no "antigen spillage". In this case, the host immune apparatus would be unaware of the presence of the tumor during the avascular period. Once the tumor was vascularized and tumor antigen poured into the circulation, it would then be too late for the immune system to react.

The best supporting evidence of this is in an article by Bray et al., International Journal of Cancer 16: 607-615, 1975, "Early Onset of Serum Blocking in a Murine Melanoma Model". After they injected 1×10^7 cells subcutaneously in mice, they were able to detect "blocking factors" in the serum as early as one hour. The level of "blocking" increased and then declined and disappeared by 48 hours, but "reappeared when the tumor was established", i.e., 4-5 days after inoculation. The authors conclude that antigen release is the cause of "blocking".

I assume that "established" means the onset of vascularization which would explain the return of circulating antigen.

By contrast, spontaneously arising tumors, especially the majority of human epithelial tumors, are separated from the bloodstream by a basement membrane, during the long avascular (in-situ) period. This seems analogous to injecting a very small population of tumor cells, so carefully into the subcutaneous space of an animal, that there would be no antigen spillage. Therefore, the first indication of antigen in the circulation would come after the tumor was already vascularized.

I would guess that the "sneaking through" phenomenon might be somewhat difficult to reproduce from laboratory to laboratory because it would depend upon being able to give a very delicate injection of a few tumor cells subcutaneously or intradermally, so as not to spill any antigen into the circulation.

Best regards,

Sincerely yours,

Judah Folkman, M.D.

mjc

VERY LARGE TUMOR
CELL INOCULUM

Moderate tumor cell
Inoculum.

Very Small tumor cell
Inoculum ("sneaking-thru")

= level of circulating
antigen or antigen-Ab
complex.

Antigen "spill"
at time of inoculation

antigen "spill"
immunizes host.

Avascular Phase of
Tumor Growth
($\approx$ 3-5 days)

Vascularized
Tumor

antigen release continues
p vascularization.

antigen reappears p vascularization
but is not sustained
because of
immune
rejection

continuous
antigen release
immunized host.
tumor
becomes rapidly growing tumor