HETEROLOGOUS TUMOR CELLS AS IMMUNOTHERAPIC AGENTS FOR CANCER

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ABSTRACT

Background
Most research studies on cancer immunity are based on the usage of modified irradiated (Eishi et al., 1998; Daniel et al., 2001) or lysed (Mitchell, 2002; John et al., 2002) tumor cells of the same cancer cell line. Little studies have been done to show if the use of heterologous (other species) of the same cancer can help in irradiating tumor. The use of autologous cancer cells proved to have improved the survival in patients with advanced colon cancer (Wolfgang et al., 2002). This method is however rendered useless if autologous cancer cells are unavailable or difficult to obtain (e.g. brain tumor). The use of cross-species cancer cell lines, if prove to show some efficacy in anti-tumor immunity, will definitely shed some hope to cancer patients whose cancer tumors are inaccessible. This kind of therapy can also save cancer patients the pain, agony and expensive costs of having operation to remove self-cancer cells for treatment. Some researches claimed that most vaccinations, which are based on the use of a single epitope, might not induce a broadly efficacious response. Such procedures are also labor-intensive. In contrast, the use of irradiated whole tumor cell is simple and likely initiates a broad antitumor response without prior need to know the tumor antigens (Dillman et al., 2002). Other researchers yet argued that although complex whole cell-derived vaccines have given clinically superior responses compared to lysate vaccines containing well-defined antigens, such as peptides or gangliosides, well-defined vaccines are theoretically more desirable because of their reproductibility (Mitchell, 2002). The aim of this investigation is to find out if the use of human colon cancer cells can elicit an anti tumor immunity to existing colon tumor in mice and as well as to serve as a cancer vaccine. The question on whether necrotic (lysate) or apoptotic (irradiated) cells is a better mean of immunotherapy will also be investigated.

METHOD

The immunotherapy experiment was done in the way where groups of mice (n=5) were injected with live parental CT26 (5 x 10⁶/100uL PBS/mice) into the left flank on day -5. Five days later (day 0), when tumor size reached around 50mm², the control group were subcutaneously injected with 100uL of PBS in the left flank near the existing tumor while the experimental group were injected with HT29 lysate or irradiated cells of varying concentration. The mice were monitored for tumor growth.

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growth in the left flank for a total of 16-18 days. Vaccination was done by subcutaneously injecting the HT29 lysate or irradiated cells (5 x 10^6/100uL/mouse) in the left flank of the mice on day-5. Control group received no vaccination. 100uL PBS was injected into the mice instead. Tumor development was monitored for 16 days.

RESULTS

Efficacies of necrotic HT29 cells in establishing antitumor immunity

Results as shown in Fig. 1A, three groups of mice (n = 5) that received the necrotic cells treatment showed a smaller tumor growth throughout the whole experiment. Better efficacy of treatment was observed with higher concentration of HT29 necrotic cells. A decrease in tumor area was seen from day 4 to day 8. From Fig 1B, mice that were given with treatment of 5 and 7.5 million HT29 necrotic cells had much smaller percentage area increase as compared to the animals injected with PBS only. The group of mice that received an sc injection of 2.5 x 10^6 HT29 necrotic cells, however, showed similar percentage area increase as the control group. Statistical analysis showed that the tumor area difference between the control group and the group treated with 2.5 million necrotic cells was not significant and that of the control group and mice that were injected with 5 or 7.5 x 10^6 HT29 necrotic cells were statistically significant.

Efficacies of irradiated HT29 cells in establishing antitumor immunity

Results as shown in Fig 2A, the two groups of mice (n = 5) that were injected with 8000 Rads irradiated cells (5 x 10^6, 7.5 x 10^6 HT29 cells) showed no difference in tumor growth with the mice that received no irradiated cell treatment. The percentage area increase in the three groups also showed similar results. Statistical analysis showed that the difference between the control and the two experimental groups were not significant.
Fig 1A,B Efficacies of irradiated HT29 cells in establishing antitumor immunity. Development of tumor (A) and percentage increase (B) of mice injected with HT29 irradiated cells 5 days after subcutaneous implantation of CT26 tumor cells.

Use of heterologous tumor cells as tumor vaccine

Results as shown in Fig 3A, all mice vaccinated with irradiated HT29 cells or HT29 necrotic cells on day -5, developed tumor after the mice were subcutaneously injected with parental live CT26 cells (5 x 10⁶) into the right flank. The tumor growth of the mice vaccinated with HT29 necrotic cells was rather similar with that of the control group that received no vaccination. Mice that were vaccinated with the irradiate HT29 whole cells were associated with smaller tumors size as compared with the control group as well as the group receiving necrotic cells vaccination. From Fig 3B, the percentage area increase of the tumor size for the mice vaccinated with HT29 necrotic cells is similar with that of the mice receiving no vaccination. Mice that were vaccinated with irradiated HT29 cells had smaller percentage area increase than those of the control animals and the necrotic cells-vaccinated mice. Statistical analysis, however, showed that these differences between the irradiated HT29 cell vaccinated animals and the non-vaccinated animals were not significant.

Fig 1A,B Use of heterologous tumor cells as tumor vaccine. Development of tumor (A) and percentage increase (B) of mice vaccinated with HT29 irradiated cells or necrotic cells 5 days before subcutaneous implantation of CT26 tumor cells.
The data presented in this paper demonstrate that HT29 lysate cells (necrotic cells), although fail in elimination of existing tumors, aid in slowing the growth of the CT26 tumor. The results also show that the irradiated (apoptotic) tumor cell treatment totally fails in control the size of the existing tumor. The necrotic cells prove to elicit a better immunity against existing tumor as compared to apoptotic cells. Tumor-associated antigens are largely differentiation antigens encoded by normal genes (Nanda and Sercarz, 1995; Wolfgang et al 2002). These proteins and corresponding peptides are true self-antigens and the autoimmune responses elicited against them are autoimmune responses. An organism uses several mechanisms to avoid such autoimmune responses. This explains the absence or weak immune responses to tumor-associated antigens. The use of heterologous cancer lysate is more immunogenic to the host as compared to synergetic cells, thereby eliciting a greater humoral immune response. The boost in the immune response is however non-specific. Anti-tumor actions are mediated by complement and antibody, macrophages and natural killer cells, all of which are non specific. Specific cell-mediated responses such as antibody-dependent cytotoxicity and direct destruction by cytotoxic T lymphocytes (CTL) are not generated. This explains the failure of irradiated HT29 cells in controlling the growth of the existing CT26 tumor as well as preventing tumor growth after vaccination.

REFERENCES