

Bacteria Used in Treatment of Cancer

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Can bacteria, instead of chemotherapeutic agents be used to more effectively treat different types of cancer?

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Currently the most common type of cancer treatment involves the use of chemotherapeutic agents. Chemotherapeutic agents are chemical agents that act by killing cells that divide rapidly. The problem with chemotherapeutic agents is that they attack all cells good and bad. This means that chemotherapy also harms cells that divide rapidly under normal circumstances: such as the cells in the [bone marrow](#), [digestive tract](#), and [hair follicles](#). Killing these cells which are essential to the normal functions of the body result in the most common side-effects of chemotherapy: [myelosuppression](#) (decreased production of blood cells, hence also [immunosuppression](#)), [mucositis](#) (inflammation of the lining of the digestive tract), and [alopecia](#) (hair loss). Aside from side effects studies currently show that fewer than 30% of those patients with relapse attain disease-free remissions after a second treatment. Scientists are now looking for ways to provide treatment to cancer patients that will cause less side effects and provide a more direct mechanism to target and kill the cancer cell. Current research has provided evidence that alternative tumor-specific strategies using cytokines, which are small cell signaling protein molecules that are secreted by numerous cells and are used for intercellular communication. Current studies focus on cytokines such as replicating bacteria, soluble receptors, and monoclonal antibodies are in fact being shown to cure patients with cancer.

Introduction

The first patient with cancer to be purposefully infected with bacteria was treated by German physician @ Bush in 1868. Nearly 30 years later, William B Coley, a young surgeon at New York Hospital, encountered a patient who seemed to be cured by a severe *Streptococcus pyogenes* infection. This observation led Coley to begin treating cancer patients with living bacteria. (Lee) One strong point of bacterial therapy is the ability to specifically target tumor sites.

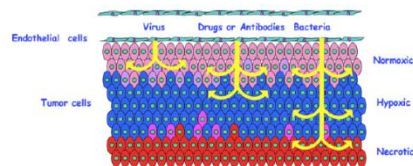
Recent Progress

The problem with chemotherapeutic in vitro cancer treatment is although it is easy to induce cell death it is nearly impossible to spare normal cells. This is because solid tumors are seldom homogenous, they have different microenvironments that often lack oxygen and a sufficient blood supply. The lack of oxygen and blood supply in

aggressive tumors creates an acidic and nutrient deprived environment. These hypoxic regions of tumors are less sensitive to ionizing radiation because its effects depend on oxygen; they are also less sensitive to chemotherapeutic agents because these drugs delivered to these regions may be suboptimal. Hypoxic regions are also associated with a more malignant phenotype, such as apoptosis, angiogenesis, and metastasis. Some anaerobic and facultative anaerobic bacteria have been used experimentally as anticancer agents because of their selective growth in hypoxic regions of solid tumors after systemic administration. Bacteria can actively move away from the vasculature and penetrate into necrotic regions of the tumors. In 1964 using *Clostridium* as an obligate anaerobic bacterium, when injected it into the body, the spores replicated and developed only in hypoxic regions. In hosts with advanced cancer, these hypoxic regions can still be

targeted since *Clostridium* develops and proliferates in oxygen-poor areas. (Lee)

Fig. 1 Schematic representing the advantage offered by bacteria as an antitumor agent. Bacteria offer several advantages like (1) tumor-targeting potential, (2) mobility, (3) transgene capacity, and (4) cheap



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Although most patients with Hodgkin's disease can be cured by a standard chemotherapy, fewer than 30% of those patients with relapse attain disease-free remissions after a second treatment. (Barth). Even though treatment using immunotoxins in most cases is effective, in some cases neutralizing antibodies often developed within three weeks and these antibodies almost always react with PE38 to limit the number of treatment cycles that can effectively be administered.

Another limitation of chemotherapeutic cancer therapy is lack of selectivity to tumor cells. Based on current research it is possible to develop a more effective therapy for patients with Hodgkins Lymphoma and other cancers by specifically targeting and killing a cancer cell without affecting other cells within the body?

In 1998 Stefan Barth, Barbel Matthey, Michael Hun, Volker Diehl and Andreas Engert proved a new recombinant immunotoxin based on the CD30 ligand is possible for use against human lymphoma." In their published article they explain that "Recombinant DNA technology makes it possible to genetically fuse V genes or cytokines to toxin domains, resulting in immunotherapeutics for selective destruction of tumor cells."

To test this theory the scientists developed a new CD30 ligand-based fusion toxin (CD30L-ETA) and ligated it into a plasmid and fused it to a modified *Pseudomonas aeruginosa* exotoxin a(ETA). After IPTG-induced expression in *E. coli* strain BL21(DE3), the 60 kDa his-tagged fusion protein was isolated and renatured in the presence of arginine and a glutathione redox system. The refolded protein was purified and concentrated. The scientists used competitive ELISA immunohistochemical staining and FACS to evaluate the binding properties of CD30L-ETA. The *in vitro* toxicity of the fusion protein was tested on the CD30 Hodgkin-derived cell and the Burkitt's lymphoma cell line BL38. It was reported that CD30L-ETA exhibited specific cytotoxicity against L540 cells. This study was the first report on the specificity and cytotoxic potency of a Chimeric CD30L fusion toxin against Hodgkins disease derived cells. (Barth).

Since this study, *Pseudomonas aeruginosa* has continued to be used as an immunotoxin to target specific cancer

cells. In 2006 a group of scientists characterized the B Cell Epitopes associated with a truncated form of *Pseudomonas* Exotoxin (PE38), which is an exotoxin used to make immunotoxins for the treatment of cancer patients. The focus of the research was to develop recombinant immunotoxins for the treatment of cancer. The agents developed are composed of 38- kDa portion of *Pseudomonas* exotoxin A (PE38), and the Fv portion of a mAb genetically fused to it. The binding activity of the Fv moiety targets the immunotoxin to AG-positive cells which are killed by the cytotoxic activity of the toxin moiety. When immunotoxins are administered to patients, neutralizing antibodies often develop within three weeks. These antibodies which almost always react with PE38 and very infrequently with the Fv, limit the number of treatment cycles that can be given. It is fortunate however that patients with certain leukemias and lymphoma infrequently make antibodies to the immunotoxin and can receive the benefit of many cycles (Onda).

This non responsiveness probably results from damage to the immune system, either due to the previous chemotherapy or because the leukemia causes immune suppression. The result of this study shows that the greatest success is with the treatment of leukemia patients. The results state that over half of the patients with life-threatening drug-resistant hairy cell leukemia achieved a complete remission after receiving three or more cycles of the immunotoxin BL22 targeted to CD22. This result suggests that immunotoxin therapy is more likely to be successful in multiple cycles of treatment can be given. This study mentions that several approaches have been proposed to decrease the immunogenicity of foreign proteins such as PE38. One type involves shielding the protein from the immune system by conjugating high molecular weight polyethylene glycol to the immunotoxin.

To do this it is required that the PEG is attached at locations on the toxin that do not identify and remove epitopes of the T cells or B cells by site mutagenesis. Previous research has provided evidence that human and mouse antibody recognize the same epitopes on foreign proteins. Using this information the scientists made a large panel of mouse anti-PE38 m, and demonstrated that antibodies to these epitopes are also present in human sera from immunotoxin-treated patients. To test this theory several mice were injected with several PE38-based immunotoxins and derived hybridomas from responding B cells. (Onda)

Antibodies were then mapped to the surface of PE38. Experiments indicate the presence of 7 major epitopes which were also recognized by human antibodies induced patients treated with immunotoxins. Among the groups epitope Ela was found to be the major neutralizing epitope and included some of the highest binding affinity antibodies. The computer analysis and mapping

experiments used a series of point mutations to confirm that the location of each epitope was clustered and not random. (Onda).

The discussion of Onda's experiment states that the successes of this experiment have focused attention on the foreignness of proteins as a major obstacle to achieving success in the clinic. The scientists present evidence that the presence of a limited number of epitope clusters on the surface of a bacterial protein PE 38, suggest that a foreign protein may be made less immunogenic by replacing structures that are likely to be epitopes with those that are unlikely to become epitopes. (Onda)

Discussion

Current research is focused on discovering and developing anticancer agents that are selectively target only tumor cells and do not harm normal cells. This is done using obligate or facultative anaerobic bacteria that are able to multiply selectively in tumors and inhibit their growth and immunotoxins. Based on the three studies discussed one can conclude that although there is still work to be done in order to maximize the effectiveness of the treatment of certain cancers with immunotoxins and bacteria, current research in this area has already proven that this type of therapy is more effective than chemotherapy on Hodgkins and lymphoma. Using immunotoxin and bacteria instead of chemotherapeutic agents is a more direct approach to target and kill cancer cells, without damaging other live and healthy cells.

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