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mAb-Alliinase conjugates produce *in situ* cytotoxic Allicin molecules from Alliin which kill tumor targeted cells

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Objectives of Research

Allicin (diallylthiosulfinate), the biologically active molecule, is produced by the enzyme allinase which condenses two molecules of the substrate alliin upon crushing of garlic cloves. Allicin has been shown to be cytotoxic to many types of cells, especially microorganisms, but also to mammalian including different types of cancer cells. One of the biggest problems facing the use of Allicin is its very high reactivity, sensitivity and short half life. Our aim is to harness and target the continuous production of Allicin molecules so as to utilize their cytotoxic effects only against desired tumor cells.

Recent findings

Killing of cancer cells by mAb-Alliinase conjugates

1. A number of monoclonal antibodies against a variety of tumor-cell specific antigens are currently being used in the clinics as an immunotherapy against different types of cancer. A conjugate consisting of the pure enzyme Alliinase chemically ligated to a mAb specific for the ErbB2 tumor antigen was prepared. This novel hybrid molecule conserved the enzymatic activity of Alliinase and the antigen recognition specificity of the antibody. Addition of solutions containing the substrate Alliin produced detectable Allicin molecules which specifically killed the ErbB2 containing tumor cells in a dose-dependent manner but did not kill normal cells devoid of the tumor receptor. Mice implanted (s.c.) with cells of a human gastric tumor cell line were injected (i.v.) with the mAb-Alliinase conjugate and then with repeated injections (i.p.) of the inert substrate Alliin. A drastic inhibition of tumor development was observed in mice that received both the conjugate and Alliin, whereas in mice that received only the conjugate or the mAb alone, there was no inhibition.

2. B-Chronic Lymphocytic Leukemia (B-CLL) is a quite prevalent disease for which there is no long lasting effective therapy. B-CLL cells display low levels of the CD-20 specific tumor antigen but the anti-CD-20 human mAb, Rituximab®, which is clinically used as a therapy against many CD-20 expressing lymphomas, has a very limited effect on B-CLL cells. Conjugates were prepared by chemically ligating Alliinase with Rituximab® and in the presence of Alliin the conjugates were shown to kill B-CLL cells obtained from patients by inducing apoptosis as detected by annexin staining. The human-mouse radiation chimera, developed by Prof. Y. Reisner (WIS), was used to demonstrate *in vivo* a high anti-B-CLL cell activity of the Rituximab-Alliinase conjugate upon Alliin administration.

In collaboration with Prof. A. Berrebi (Kaplan Hospital, Israel), B-CLL cells were obtained from advanced stage patients and engrafted (i.p) in the Hu-mice chimeras. The conjugate was injected and after a few days it was followed by repeated injections of Alliin. Cells were then extracted from the peritoneum and analyzed for viability with a fluorescence activated cell sorter. A very significant killing of B-CLL cells was observed following 4 administrations of Alliin (>70 %) but no anti-tumor effect was observed in the absence of alliin or with the Rituximab® alone. The same conjugate was shown to display also very good anti-tumor activity both *in vitro* and *in vivo* in the same chimeric mice model against a number of lymphoma cell lines that express surface CD20, such as an EBV-transformed cell line and a Mantle Cell Lymphoma (MCL) line.

3. Killing of Aspergillus and Candida spp. by an anti-fungal mAb-alliinase conjugate

Life threatening fungal infections by *Aspergillus* and *Candida spp.* are quite often seen in immunocompromised persons, especially in patients after bone marrow transplantations. Most

of the available anti-fungal drugs have considerable toxicity and resistance to most drugs is becoming more prevalent. Allicin was found to have a very potent anti-fungal activity at very low concentrations (< 1 μM) against a variety of *Aspergillus* and *Candida spp.*. In collaboration with Dr. Nir Osherov (TAU, Tel Aviv) we directly injected (i.v. 4 x times) pure allicin to mice infected with conidia of *Aspergillus fumigatus* isolated from patients. Such treatment significantly prolonged the survival of the infected mice and markedly reduced their kidney fungal load. The efficacy of an *in vivo* treatment with a conjugate consisting of an anti-Aspergillus mAb conjugated to Alliinase and Alliin against a fungal infection in a mouse model is currently being assessed.

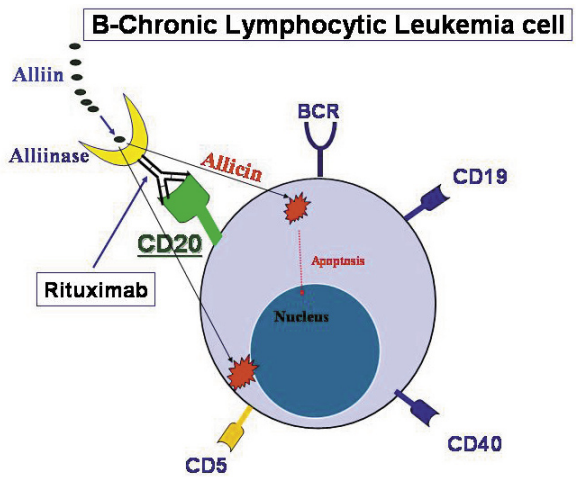

Fig. 1 *Model of B-CLL cells and their interaction with the RituximAb-alliinase conjugate and Alliin*

The anti-hypertensive effect of Allyl-mercapto-captopril

In previous work together with Prof. Talma Rosenthal and Dr. Edna Peleg (TAU, Med. School) we have shown that daily oral administration of Allicin significantly lowered both the blood pressure and tryglycerides in the rat hypertensive animal model. The novel and stable compound Allyl-mercapto-captopril which was produced by reacting the known anti-hypertensive and free thiol containing molecule - Captopril®, with allicin, was found to lower blood pressure and triglycerides in hypertensive rats at significantly smaller doses than those used for Captopril®.

Selected Publications (from 2002)

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