The use of chimeric bacterial and plant protein toxins for targeted chemotherapy

Clin Microbiol Infect 1998; 4: 357–358

The immune substances . . . in the manner of magic bullets, seek out the enemy
Paul Ehrlich

The principle of targeting drugs to specific cells as weapons for selective killing of malignant and other disease-causing cells goes back nearly 100 years. In 1906, Paul Ehrlich predicted the construction of 'magic bullets' (*Zauberkugeln*) as 'bodies which possess a particular affinity for a certain organ . . . as a carrier by which to bring therapeutically active groups to the organ in question' [1].

Over the past 25 years, the confluence of the discovery and applications of monoclonal antibodies, the progress in protein toxin research, the applications of recombinant DNA methodologies, and genetic and protein engineering, have allowed Ehrlich's goal to be realized.

In this respect, a great number of experimental and clinical investigations were and are still developed with chemically or genetically engineered chimeric (hybrid) molecules in which various cell-binding ligands (particularly immunoglobulins) were coupled to highly cytotoxic protein toxins (or their subunits) to generate chemotherapeutic agents to specifically kill malignant, virus-infected (e.g. HIV, HTLV) or other potentially pathogenic host cells (see references 2–9 for reviews).

Toxin moieties of the conjugates

Many protein toxins of bacterial or plant origin endowed with potent cytotoxic properties towards eukaryotic cells have been evaluated. The most commonly used bacterial toxins to generate appropriate hybrids for experimental purposes and/or clinical trials are diphtheria toxin [4–8,10–13] and *Pseudomonas aeruginosa* exotoxin A [4–7,14,15].

Both toxins kill target cells by the same molecular mechanism. After binding to host cells they are internalized and their respective active moieties (fragment A) are translocated into the cell cytosol, where they ADP-ribosylate elongation factor 2 (EF2) and thereby inhibit protein synthesis, leading to cell death [5–7].

The plant toxins ricin and to a lesser extent abrin have also been used [6,8,16–19]. These two-chain toxins inhibit protein synthesis by inactivating the 28S RNA of eukaryotic cells [4–6]. The ricin-like single-chain gelonin was also used as a chimeric conjugate for in vivo treatment of experimental Heymann's nephritis in rats [20]. Another conjugate based on the membrane-damaging toxin perfringolysin O was also used as an experimental cell killer [21].

Cell-binding ligands of the conjugates

The ligands to be coupled to the bioactive toxin moieties belong to various families. The most commonly used targeting agents since 1975 are monoclonal antibodies generated against tumor-associated antigens or certain T- and B-lymphocyte or natural killer (NK) cell surface antigens (CD2,11,18,22,30,33). These hybrids were designed to kill hematopoietic and tumor cells or HIV-infected T-cells, or to treat autoimmune diseases (e.g. rheumatoid arthritis, diabetes) [7,8]. The antibody-toxin conjugates constitute the so-called immunotoxins (IT). The first generation of ITs was composed of whole IgG antibodies chemically conjugated to the above mentioned protein toxins. Due to the large size of the antibody molecule (150 kDa), poor tumor penetration was obtained. This and other problems were overcome by the design of the second-generation ITs based on antibody engineering and recombinant DNA techniques with a concomitant increase in tumor penetration potential. These novel recombinant ITs contained only the 25-kDa Fv heterodimer of the heavy and light chains of the antibody conjugated to the appropriate toxin moiety by either a peptide linker (ScFv) or a disulfide bond (dsFv).

Members of the other class of hybrid molecules to be targeted to disease-causing cells do not include antibodies or Fv fragments. The chimeric agents are generated by replacing (by gene fusion methodologies) the native cell-binding domains of the appropriate bacterial or plant toxins with various effectors such as: transforming growth factor-α (TGF-α), which targets epidermal growth factor receptors on many types of malignant cells; α-melanocyte-stimulating hormone (α-MSH), which targets α-MCH receptor on melanoma cells; and IL-2, IL-6, IL-15 and GM-CSF in the therapy of various hematologic malignancies and psoriasis [5,7,8,11,22,23].

Conclusions

The considerable experimental and clinical investigations based on toxin-fusion ligands are quite impressive. New improvements in the development of safe and effective

chimeric molecules will probably lead to a new class of therapeutic agents against severe diseases.

J. E. Alouf
Department of Teaching and Education,
Institut Pasteur de Lille,
Lille, France

Tel: +33 0 3 20 87 79 01 Fax: +33 0 3 20 87 72 24/73 11
E-mail: Joseph.Alouf@pasteur-lille.fr

References

1. Ehrlich P. The relationship existing between chemical constitution, distribution, and pharmacological action. In Himmelweite F, Marquardt M, Dale H, eds. The Collected Papers of Paul Ehrlich, Vol. I. New York: Pergamon, 1956: 596–618.
2. Jansen FK, Blythman HE, Carrière D, et al. Immunotoxins: hybrid molecules combining high specificity and potent cytotoxicity. Immunol Rev 1982; 62: 185–216.
3. Byers VS, Baldwin AW. Therapeutic strategies with monoclonal antibodies and immunoconjugates. Immunology 1988; 65: 329–35.
4. Olsnes S, Sandvig K, Peterson OW, van Deurs B. Immunotoxins—entry into cells and mechanisms of action. Immunol Today 1989; 10: 291–5.
5. Murphy JR, Williams DP. Protein engineering of microbial toxins: design and properties of novel fusion proteins with therapeutic and immunogenic potential. In Alouf JE, Freer JH, eds. Sourcebook of bacterial protein toxins. London: Academic Press, 1991: 491–505.
6. Vitetta ES, Thorpe PE, Uhr JW. Immunotoxins: magic bullets or misguided missiles. Immunol Today 1993; 14: 252–8.
7. Brinkmann U. Recombinant immunotoxins: protein engineering for cancer therapy. Mol Med Today 1996; 10: 439–46.
8. Trush GR, Lark LR, Clinchy BC, Vitetta ES. Immunotoxins: an update. Annu Rev Immunol 1996; 14: 49–71.
9. Chiron MF. Immunotoxines recombinantes et toxines chimères pour une thérapie ciblée en oncologie. Bull Cancer 1997; 84: 1135–40.
10. Aullo P, Alcani J, Popoff MR, Klatzmann DR, Murphy JR, Boquet P. *In vitro* effects of a recombinant diphtheria-human CD4 fusion toxin on acute and chronically HIV-1 infected cells. EMBO J 1992; 11: 575–83.
11. Gottlieb SL, Gilleaudeau P, Johnson R, et al. Response of psoriasis to a lymphocyte-selective toxin (DAB_{389} Il-2) suggests a primary immune, but not keratinocyte, pathogenic basis. Nature Med 1995; 1: 442–7.
12. LeMaistre CF, Craig FE, Meneghetti C, et al. Phase I trial of a 90- minute infusion of the fusion toxin DAB_{486} IL-2 in hematological cancers. Cancer Res 1993; 53: 3930–4.
13. Tepler I, Schwartz G, Parker K, et al. Phase I trial of an interleukin-2 fusion toxin (DAB486IL-2) in hematologic malignancies: complete response in a patient with Hodgkin's disease refractory to chemotherapy. Cancer 1993; 73: 1276–85.
14. Pai LH, Wittes R, Setser A, Willingham MC, Pastan I. Treatment of advanced solid tumors with immunotoxin LMB-1: an antibody linked to *Pseudomonas* exotoxin. Nature Med 1996; 2: 350–3.
15. Debinski W, Puri RK, Kreitman RJ, Pastan I. A wide range of human cancers express interleukin-4 (IL4) receptors that can be targeted with chimeric toxin composed of IL-4 and *Pseudomonas* exotoxin. J Biol Chem 1993; 268: 14065–70.
16. Engert A, Diehl V, Schnell R, et al. A phase-I study of an anti-CD25 ricin A-chain immunotoxin (RFT5-SMPT-dgA) in patients with refractory Hodgkin's lymphoma. Blood 1997; 89: 403–10.
17. Lynch TJ, Lambert JM, Coral F, et al. Immunotoxin therapy of small-cell lung cancer: a phase I study of N901-blocked ricin. J Clin Oncol 1997; 15: 723–34.
18. Amlot PL, Stone MJ, Cunningham D, et al. A phase I study of an anti-CD22 deglycosylated ricin A chain immunotoxin in the treatment of B-cell lymphomas resistant to conventional therapy. Blood 1993; 82: 2624–33.
19. Grossbard ML, Lambert JM, Goldmacher VS, et al. Anti-b4-blocked ricin—a phase-I trial of 7-day continuous infusion in patients with B-cell neoplasms. J Clin Oncol 1993; 11: 726–37.
20. Misquith S, Surolia A. In vivo treatment of Heymann's nephritis using a cytotoxic protein–toxin conjugate. FEBS Lett 1995; 373: 151–4.
21. Drobniewski FA, Watson GJ, Wawrzynczak EJ, Alouf JE, Thorpe PE. A novel membrane-acting immunotoxin, the immunolysin, with therapeutic potential. Biochem Soc Trans 1992; 20: 318.
22. Murphy JR, van der Spek J. Targeting diphtheria toxin to growth factor receptors. Semin Cancer Biol 1995; 6: 259–67.
23. Frankel AE, Hall PD, Burbage C, et al. Modulation of the apoptotic response of human myeloid leukemia cells to diphtheria toxin granulocyte- macrophage colony-stimulating factor fusion protein. Blood 1997; 90: 3654–61.