Ann Hematol (2001) 80:B132–B134

SELECTED PAPERS

Freda K Stevenson · Delin Zhu · Jason Rice

New strategies for vaccination and imunomodulation in NHL

Abstract Knowledge of the genetic changes which occur in cancer cells is stimulating research aimed towards new therapies. Immunotherapeutic approaches, particularly antibody therapy, are already finding a place in treatment of hematological malignancies. Vaccination will build on experience in the field of infectious diseases, and it should be possible to design vehicles to deliver the expanding range of tumour antigens to the immune system. For DNA vaccines, fusion genes have the potential to activate and direct immune effector pathways. One candidate antigen for B-cell malignancies is the clonal idiotypic immunoglobulin and we have designed a fusion vaccine encoding idiotypic sequence fused to a sequence from a powerful antigen from tetanus toxin. This promotes protective immunity against lymphoma in models, and is now in clinical trial. One challenge is to bring patients into remission without significant damage to immune capacity. Another is to rethink the nature of clinical trials so that more pilot studies of efficacy can be carried out. There is no evidence so far of toxicity due to injection of DNA, but for antigens which are expressed by normal cells, the line between attack on tumour and autoimmunity will have to be carefully drawn.

Keywords B-cell tumors · DNA vaccines · immunotherapy · Idiotypic immunoglobullin · Variable region genes

Introduction

The information being generated by the sequencing of the human genome is offering opportunities to develop new ways of treating cancer. One rapidly developing area is in manipulation of the immune response to attack cancer cells. This approach is particularly attractive for those cancers, such as non-Hodgkin's lymphoma, where patients can achieve remission after initial chemotherapy, but where residual tumour cells can emerge and cause relapse. Genetic information is contributing in three main areas: first, in identifying cancer-related molecules which distinguish the cancer cell from normal cells; second, in improving our understanding of immune mechanisms which can be mobilized to attack cancer cells; third, in the development of gene-based delivery systems for vaccines to treat patients (Fig.1).

F.K. Stevenson (✉)
Molecular Immunology Group, Tenovus Laboratory, Southampton University Hospitals Trust, Southampton SO16 6YD, UK
e-mail: fs@soton.ac.uk
Tel.: +44 2380 796923, Fax: +44 2380 701385

Target antigens on lymphoma cells

Techniques to identify molecules, which may be mutated or over-expressed in cancer cells, are now available. Our focus is on lymphoma, predominantly a tumour of B lymphocytes, and Fig. 2 shows the various categories of potential tumour antigens. Some tumours are known to harbour viruses, such as Epstein-Barr virus, and viral antigens could be considered as vaccine targets either in a preventative or treatment setting. Interestingly, 10–20% of human cancer has a known viral association, with one example being hepatitis B virus and hepatocellular carcinoma. In Taiwan, vaccination against hepatitis B is already leading to a reduction in incidence of liver cancer [2]. Other lymphoma-associated antigens arise from chromosomal translocations, from mutated proto-oncogenes, and from proteins arising due to general genetic instability. The new microarray technology, which can survey gene expression in tumour cell populations, is likely to reveal many new candidate tumour antigens. Tumour cells may also have aberrant expression of molecules such as mucins, due to changes in glycosylation. Certain molecules, termed "cancer-testis" proteins, usually expressed only in the testis, may become expressed in tumours due to hypomethylation. Together with proteins produced by specialized cells such as the clonal immunoglobulin of B cell tumours, this vast and increasing number of tumour antigens [5] awaits the development of delivery systems to activate immunity.

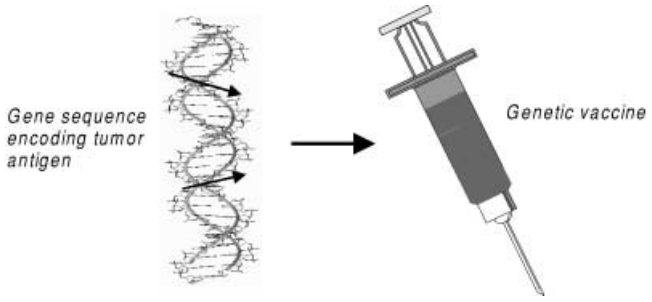

Fig. 1 Immune attack on cancer by turning genes into vaccines

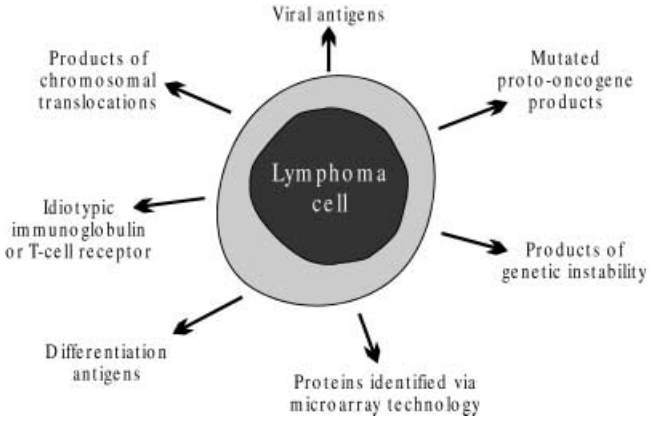

Fig. 2 Candidate tumour antigens expressed by lymphoma cells potentially able to act as targets for immune attack

DNA vaccines

Once a candidate cancer-associated molecule has been identified, the gene sequence can be placed directly into a DNA vaccine format, thereby avoiding the need to make and purify the protein. A DNA vaccine is a simple vehicle, consisting of a backbone of bacterial DNA into which the cDNA encoding the potential antigenic sequence is inserted. Transcription is usually driven by a promoter/enhancer derived from CMV. There is a widespread interest in using DNA vaccines to induce preventative immunity against infectious organisms, and the experience gained in those studies has relevance for cancer vaccines [8] Following injection, the encoded protein is synthesized by the patient in a form aimed to induce specific immunity. The bacterial DNA also contributes to activation of immunity, due to the presence of immunostimulatory bacteria-specific sequences in the backbone able to stimulate the innate immune response [12]. The effect of these is to release mediators including IFN-γ. IFN-γ, IL-12 and IL-18 from macrophages/monocytes, and IFN-γ from NK cells.

DNA vaccines are usually injected into either muscle or skin, and transfected muscle cells or keratinocytes are likely to act as antigen depots [4] Uptake by the "professional" patrolling cells which will present antigen to T lymphocytes, and initiate the immune response then occurs. There is also evidence for direct transfection of the antigen-presenting cells, including dendritic cells, especially from the skin site where Langerhans cells are in abundance [3] The outcome is induction of antibody and T-cell responses against the encoded protein, with a bias towards a T_H1–dominated response due to the cytokines released by the innate immune system. DNA vaccines are also very effective in inducing cytotoxic T-cells, which can then directly kill antigen-positive cells, including tumour cells. For cancer, vaccination is necessarily largely aimed at treatment rather than at prevention. The challenge then is to activate immunity capable of attacking cancer cells on a continuing basis, in patients who may have a weakened or damaged immune capacity. To succeed, we need to ensure that tumour antigens which have failed to activate effective immunity when present in cancer cells are presented from the DNA vaccine in a form which engages the immune response.

DNA vaccines against lymphoma

The first tumour antigen we chose to test in a DNA vaccine was the clonal immunoglobulin expressed by the majority of B-cell lymphomas. The function of this Ig in normal cells is to recognize foreign antigen, and this is achieved by a process of recombination and somatic mutation of the Ig heavy and light chain variable region genes, V_H and V_L, during B-cell maturation. When a B-cell tumour develops, the clonal Ig remains, and the unique sequences encoded by the V-genes can then be considered as tumour antigens, expressed as idiotypic (Id) determinants [9]. We already knew that Id protein isolated from lymphoma cells, could be used to successfully vaccinate against lymphoma, both in mouse models [7] and in patients [6]. However, preparation of Id protein on an individual patient basis is technically demanding and expensive, and this led us to investigate DNA vaccines as an alternative.

We isolated the V-genes from a lymphoma model and assembled the genes as single chain Fv (scFv), a convenient format for making protein which expresses Id determinants similar to those in the original Ig [9]. However, perhaps unsurprisingly, scFv sequence alone was completely unable to active anti-Id immunity [9]. We considered that the reason for this was that scFv, essentially an autologous protein, was not sufficiently interesting to the immune system. To make it more interesting, we attached a gene encoding a pathogen-derived protein to the 3'-end of the scFv. We chose a gene which encodes a fragment (Fragment C (FrC)) of tetanus toxin, and this dramatically enhanced the immune response against the attached scFv sequence [7] It also led to protective immunity against lymphoma [7]. The scFv-FrC fusion gene design is now in a clinical trial of patients with lymphoma.

DNA fusion gene vaccines

The reason that the scFv-FrC fusion design is so effective is likely to be due to several factors. The immune system

has developed to recognize infectious organisms, and the presence of a pathogen-derived sequence is likely to act as an alert or danger signal. In addition, mobilization of helper T cells, able to recognize the FrC, and provide the cytokine ingredients required to activate immunity against the scFv, occurs with the fusion protein. We know that fusion is necessary, since separate plasmids are ineffective [7]. For lymphoma, where Id antigen is displayed at the cell surface, anti-Id antibody is a strong mediator of protective immunity, and the fusion gene amplifies anti-Id very strongly [7]. However, we have also found that the scFv-FrC fusion design induces protective immunity against a myeloma model where Id antigen is secreted but not expressed [7]. In myeloma, protection appears to be T-cell mediated.

Importantly, we have found that the fusion gene design amplifies antibody responses against other potential tumour antigens such as carcinoembryonic antigen or MUC1 mucin [10] This indicates that the approach may have wide application. However, while fusion is likely to be a requirement, FrC may not be the only gene with promotional activity. Although pre-existing immunity to tetanus toxoid appears not to inhibit responses to the fusion vaccine [7, 10], we are also investigating alternative pathogen-derived genes. Other groups are using xenogeneic Ig sequences [11] or chemokines [1] as promoters of anti-tumour responses. DNA plasmids can also be used to deliver additional molecules, such as growth factors or cytokines, able to stimulate or direct immune reactivity against chosen tumour antigens. It should be emphasized that target antigens come in several forms, either as surface glycoproteins, secreted proteins, or as peptides in association with MHC Class I or II molecules. Vaccine design can be manipulated to stimulate the appropriate effector mechanism for each form, with T cells required for MHC-associated peptides. Recently we have designed a DNA vaccine containing a minimal sequence from FrC fused to a MHC Class I-binding peptide. This design is able to induce strong cytotoxic T-cell activity against tumour-specific peptides. We predict that a double vaccine approach, directed against two candidate tumour antigens will be highly effective in eliminating cancer cells.

Conclusions

DNA vaccines provide a promising and convenient vehicle for delivering encoded tumour antigens to sites able to activate the full range of immune pathways. The ease of manipulation and relatively low cost of construction mean that assembly and testing of vaccines can be rapid. There is a myriad of opportunities to add activating molecules either within the vaccine plasmid, or via separate plasmids.

DNA vaccines can be patient-specific, and for some cancers, disease-specific. We envisage DNA cassettes for each situation, consisting at present of tumour-derived genes fused to FrC. It should be possible to target a wide range of cancers, and the ideal setting for vaccination is clinical remission. At this stage, patients have only low levels of persisting cancer cells,.but should retain immune capacity. However, cancer cells are slippery targets, able to modulate or delete expression of many potential antigens when under immunological pressure. To avoid this, we plan to use a double vaccine approach which targets two antigens at the same time.

Vaccination against infectious diseases has been a highly successful intervention in the field of public health. Since Edward Jenner introduced vaccination against smallpox more than 200 years ago, the impact of vaccination on the incidence of a wide range of infections has been dramatic. However, it took about 200 years to totally eliminate smallpox, and the next target virus, polio, is slowly disappearing only now. It will take time for “designer” vaccines to have an impact on the treatment of cancer, but an exciting beginning has been made.

References

1. Biragyn A, Tani K, Grimm MC, Weeks S, Kwak LW (1999) Genetic fusion of chemokines to a self tumor antigen induces protective, T-cell dependent antitumor immunity. Nature Biotechnology 17:253–258
2. Chang MH, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, Liang DC, Shau WY, Chen DS (1997) Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. New England Journal of Medicine 336: 1855–1859
3. Condon C, Watkins SC, Celluzzi CM, Thompson K, Falo LD Jr (1996) DNA- based immunization by in vivo transfection of dendritic cells. Nature Medicine 10:1122–1128
4. Davis HL, Brazolot Millan CL, Watkins SC (1997) Immune-mediated destruction of transfected muscle fibers after direct gene transfer with antigen-expressing plasmid DNA. Gene Therapy 4:181–188
5. Henderson RA and Finn O. (1996) Human tumor antigens are ready to fly. Advances in Immunology 62:217–256
6. Hsu F, Caspar CB, Czerwinski D, Kwak LW, Liles TM, Syrenglas A, Taidi-Laskowski B, Levy R (1997) Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma-long-term results of a clinical trial. Blood 89:3129–3135
7. King CA, Spellerberg MB, Zhu D, Rice J, Sahota SS, Thompsett AR, Hamblin TJ, Radl J, Stevenson FK (1998) DNA vaccines with single-chain Fv fused to fragment C of tetanus toxin induce protective immunity against lymphoma and myeloma. Nature Medicine 4:1281–1286
8. Liu MA, Fu TM, Donnelly JJ, Caulfield MJ, Ulmer JB (1998) DNA vaccines.Mechanisms for generation of immune responses. Advances in Experimental Medicine and Biology 452:187–191
9. Stevenson FK, Zhu D, King CA, Ashworth LJ, Kumar S, Hawkins RE (1995) Idiotypic DNA vaccines against B-cell lymphoma. Immunological Reviews 145:211-228
10. Stevenson FK, Zhu D, Spellerberg MB, King CA, Sahota SS, Rice J, Thompsett AR, Hamblin TJ (1999) Genetic vaccines against defined tumor antigens of B-cell malignancies. Reviews in Clinical and Experimental Hematology 9:2-20
11. Syrenglas AD, Chen TT, Levy R (1996) DNA immunization induces protective immunity against B-cell lymphoma. Nature Medicine 2:1038–1041
12. Wagner H (1999) Bacterial DNA activates immune cells to signal infectious danger. Advances in Immunology 73: 329–368