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ANTI-IDIOTYPIC DNA VACCINES FOR B-CELL LYMPHOMA THERAPY

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1. ABSTRACT

The idiotypic determinants expressed by immunoglobulin at the surface of malignant B-cells provide specific targets for vaccination strategies. However, as self-antigens they are poorly immunogenic and vaccines must include carriers to improve immune responses. Chemical cross-linking of purified idiotypic protein is so far the only method which has been employed in clinical trials while a number of second-generation vaccines have been developed in mouse models. These strategies are based on the use of recombinant DNA technology to create fusion proteins that contain the idiotype genetically linked to molecules that act as immunological adjuvants. Fusion proteins can be made available to the immune system by direct delivery of naked DNA with great advantages in terms of time and costs. The most relevant approaches are listed and discussed with particular emphasis to the mechanism by which different molecules exert their adjuvant effect. The role of cellular versus antibody mediated suppression of tumor growth following Id vaccination is examined by comparing the most recent data on the mechanism of tumor protection. In addition, an analysis of the specificity of anti-idiotypic antibodies induced by scFv-DNA vaccination as compared to protein immunization is provided.

2. INTRODUCTION

The development of therapeutic vaccines against cancer relies on the identification of defined tumor specific antigens to induce anti-tumor immunity. The immunoglobulin expressed on the surface of B-lymphoma cells represents an ideal model tumor antigen for testing vaccination strategies. The particular combination of the two V regions define the idiotype (Id), a unique antigenic structural feature that can be specifically detected by anti-Id antibodies. Since idiotypes reflect the uniqueness of each immunoglobulin molecule, and each B lymphocyte expresses a single arrangement of light and heavy chain V regions, they represent clonal signatures of individual B cells. During neoplastic transformation of a B cell clone, the idiotypic determinants are preserved, providing a useful tumor specific antigen to be attacked by immunotherapy.

The ability of purified idiotypic protein to induce idiotype specific resistance to tumor growth was shown to be effective in several murine lymphoma models. The lymphoma immunoglobulin itself appeared poorly immunogenic but induced high titers of anti-Id (anti-idiotypic) antibodies and protection against tumor challenge when linked to a strong immunological carrier (keyhole limpet hemocyanin: KLH) (1, 2). These pioneering studies demonstrated that, although nominally self-antigens, idiotypic determinants can become immunogenic when administered in a context that allows to overcome T-cell tolerance. The need to avoid chemical cross-linking and adjuvants that may have deleterious side effects prompted the search for alternative ways to enhance idiotype immunogenicity. Cytokines as immunomodulatory molecules were tested and their efficacy compared in protein immunization studies. Collectively these studies showed that GM-CSF, a pleiotropic cytokine with known effects on proliferation and differentiation of a variety of cells, is the most potent adjuvant (3, 4). The experience gained in murine studies led to the first clinical trials of patients with follicular lymphoma (5). Recently, a promising study demonstrated that administration of patient specific idiotypic protein coupled to KLH and co-administered with GM-CSF induced clearance of residual

tumor cells and long term disease free survival (6). An alternative approach to chemical crosslinking is immunization with autologous dendritic cells pulsed with idiotypic protein. This approach has given encouraging results in two different clinical trials (7, 8). However, application of the protein vaccination approach to patients is limited by the time consuming and expensive procedures involved in preparation of individual antigenic idiotypic protein. A more feasible approach relies on molecular cloning techniques and DNA-based antigen delivery system that have already been widely tested in murine lymphoma models.

3. ANTI-IDIOTYPIC DNA VACCINES

More then a decade ago it was first demonstrated that injection of a foreign gene as plasmid DNA into mouse skeletal muscle led to persistent expression of the gene product *in vivo* (9). Since then, genetic immunization has proved to be a valuable tool to induce effective immunity against viral and neoplastic diseases (10, 11). Indeed, DNA vaccines possess some striking properties that render them attractive for a variety of applications. They are relatively easy to build offering a great advantage over protein vaccines. Since the antigen is produced *in vivo* by cells of the host it can gain access to the MHC class I presentation pathway, inducing cytotoxic responses as well. Moreover, when the immunizing gene encodes for a secreted or membrane antigen, protein synthesis in the host is more likely to give rise to a product whose conformation is similar to the wild-type protein. This may be important in the context of cell surface exposed tumor antigens, to favor the induction of conformational antibodies. Moreover, the presence of CpG unmethylated dinucleotide in the backbone of plasmid DNA has an inherent immuno-adjuvant effect inducing proliferation of splenocytes, cytokine production and maturation of dendritic cells (12, 13). Interestingly, it has been also suggested that CpG motifs can induce expression of MHC class II molecules on tranfected myocytes that became thus capable to directly activate CD4+ T cell (14).

Plasmid DNA can be delivered by needle injection (in the muscle or in the dermis) or by bombardment of DNA-coated gold particles. The latter method is particularly convenient since it requires much lower amount of DNA than the injection of DNA in solution. Following delivery of plasmid DNA antigen presentation can occur either upon internalization of antigen released by non-presenting transfected cells or by direct transfection of tissue resident DCs. The former mechanism was shown to be predominant following needle injection of plasmid DNA (15). Conversely, gene-gun bombardment can also deliver the antigen to Langherans cells resulting in general activation of dendritic cells despite the number of cells directly transfected is low (16). The routes of immunization can influence the type of immune response with needle injection at raising a predominantly Th1 responses and gene-gun bombardment a Th-2 response (17). Furthermore, several recent studies showed that it is possible to fine-tune the immune response by adding plasmids that encode for particular cytokines or chemokines in the vaccine formulation (18, 19).

Thanks to the advance in antibody engineering, the two relevant antigenic domains, VL and VH, from the lymphoma immunoglobulin can be easily cloned and assembled into an appropriate format (single chain Fv, scFv, or complete chimeric Ig) (figure 1) to be delivered directly as plasmid DNA. A scFv contains only VL and VH linked by a short flexible linker that enables both V regions to fold and associate with the same parallel orientation as in the original immunoglobulin. However, proper folding of scFv molecules and actual retention of the original idiotypic conformation is always an important issue to be addressed *in vitro* before testing the molecules *in vivo*. The scFv cassette can be cloned in plasmid expression vectors to produce the recombinant protein in bacteria or eukaryotic cells or can be delivered directly as plasmid DNA. In this last case plasmids should contain the appropriate elements for expression in the host mammalian cells.

As in the case of the idiotypic protein, initial DNA vaccination studies in murine models showed that the tumor derived V regions alone are poorly immunogenic but the relative ease to manipulate the antigen encoding cassette has facilitated the testing of several different designs to augment immunogenicity (table 1). It has been clearly demonstrated by several groups that genetic coupling of a T cell epitope carrier molecule to the scFv is a successful approach to break T-cell tolerance and to obtain high levels of anti-Id responses. Both xenogeneic (20, 21) and bacterial derived sequences (22) were shown to act as efficient T-cell help providers. Analysis of the minimal requirement to achieve optimal responses showed that both tumor derived V regions are needed for efficient tumor protection (23).

An alternative approach to convert non-immunogenic scFvs in potent immunogens is based on the specific targeting to APC. Two chemokines, IP-10 and MCP-3, were N-terminally fused to the scFv from two different lymphoma and delivered as plasmid DNA or purified proteins (24). The fusions elicited strong immune responses that protected from tumor challenge in both models and with both chemokines. The adjuvant effect is supposed to be due to targeting of the antigen to professional antigen presenting cells where the receptor for chemokines is expressed. The resulting increase of antigen uptake and presentation allows to by-pass T-cell tolerance without the need of other adjuvant molecules. This view is suggested by the requirement of a physical link between the scFv antigen and the functionally active chemokine, although a formal proof of the mechanism is still awaited. A similar mechanism has been claimed in the case of a fusion between the idiotype and cytotoxic T-lymphocyte antigen 4 (CTLA4), whose ligands (B7 molecules) are expressed on the surface of antigen presenting cells (25). However, in this study the results are less apparent because the immunogen contained also a xenogeneic region besides the CTLA4 targeting domain. In the BCL-1 lymphoma model, a similar fusion between CTLA4 and the immunogenic scFv/CH3 was unable to enhance the

Table 1. Anti-Id DNA vaccination strategies

Tumor cell line	Mouse strain	Ig expression	Vaccine formulation	Reference
38C13 B-cell lymphoma	C3H/HeN	IgM/kappa	Chimeric Ig: Id-human gamma1/kappa	20
			Chimeric Ig fused to GM-CSF	20
			ScFv fused to IL-1 beta derived peptide	35
			ScFv fused to MCP-3 chemokine	24
			Chimeric Ig Id-human gamma1/kappa in an adenovirus vector	27
A31 B-cell splenic lymphoma	CBA/Hx C57BI6F1	IgM/kappa	ScFv fused to fragment C of Tetanus toxin	22
			ScFv fused to the coat protein of Potexvirus	26
5T33M myeloma	C57BL/Kal WRij	IgG2b/kappa	ScFv fused to fragment C of Tetanus toxin	22
			ScFv fused to the coat protein of Potexvirus	26
A20 B-cell lymphoma	Balb/c	IgG/kappa	ScFv fused to MCP-3 chemokine	24
BCL-1 B-cell lymphoma	Balb/c	IgM/lambda	ScFv fused to human gamma1 CH3	23
			Chimeric Ig (Id-human gamma1/kappa) in an adenovirus vector	27

anti-idiotype specific Ab response. Moreover, a direct fusion to the non-immunogenic scFv did not induce any detectable level of anti-idiotype antibodies (F. B. and O. B., unpublished data).

Most recently, an alternative fusion gene between a scFv and a protein derived from a plant virus was described as a further way to provide T-cell epitopes and induce protective immunity in lymphoma and myeloma (26). Moreover, the power of DNA vaccines can be combined to the advantages of a viral delivery system, as demonstrated by idiotype transduced adenovirus delivery system. A single intramuscular injection of Id-encoding recombinant adenoviruses induced potent humoral and cellular response in two lymphoma models (27).

4. MECHANISM OF TUMOR PROTECTION

The evidences accumulated so far on tumor protection induced by anti-idiotypic immunization do not allow to clearly define the main mechanism involved in the rejection of tumor cells. It has been possible to induce antibody, $CD4^+$ T cells and $CD8^+$ T cells specific for the idiotype. In mouse models, both protein and DNA vaccines were shown to work mainly through an anti-idiotypic antibodies dependent mechanism (22, 27, 28, 29). An exception is represented by vaccination with scFv-chemokine fusion proteins that, according to depletion studies, protected from tumor challenge through a mechanism dependent on effector T-cells (24).

In human studies however, clinically relevant immune responses induced by immunization with Id-protein or Id pulsed dendritic cells were dependent on the induction of Id specific CD8+ T cell (6, 8). Moreover, syntetic peptides derived from immunoglobulin framework region shared among lymphoma patients were able to induce cytotoxic responses (30). Although this model should be validated by *in vivo* immunization studies, it indicates that malignant B-cell can process and present class I restricted peptides derived from immunoglobulin framework V regions.

Induction of cytotoxic T-cell responses offers the interesting perspective to extend Id vaccination to surface negative myeloma and to other type of tumors that do not express surface antigens. However, it is important to consider that recruitment of CD8+ T-cells may be stricter because it requires the presence of appropriate MHC class I epitopes within the tumor V regions. On the contrary, as long as epitopes to provide cognate T-cell help are included in the vaccine, anti-Id antibodies can always be induced. Hence, although it is conceivable that both arms of the immune system may collaborate to eliminate tumor cells following Id vaccination, promotion of antibody mediated protection could be favorable, at least for surface Ig positive lymphomas (figure 2).

In the context of antibody mediated protection, it is not fully understood whether they act by recruiting effector mechanisms such as complement and antibody mediated cytotoxicity (CDC and ADCC) (2, 28), or by direct inhibition of tumor growth by signaling through the BCR receptor (31, 32) (figure 2). This raises the question of the binding specificity of anti-idiotypic antibodies. The specificity of anti-Id antibodies has been analyzed following scFv-genetic vaccination in the BCL1 lymphoma model (21). By analyzing the binding of anti-Id antibodies to properly folded idiotypes the authors showed that anti-Id antibodies induced by scFv DNA vaccination are directed exclusively to epitopes arising from the V_L/V_H association, thus highly specific. This finding implies that anti-Id antibodies induced with constructs encoding only one V region or part of it (33, 34) may be less specific.

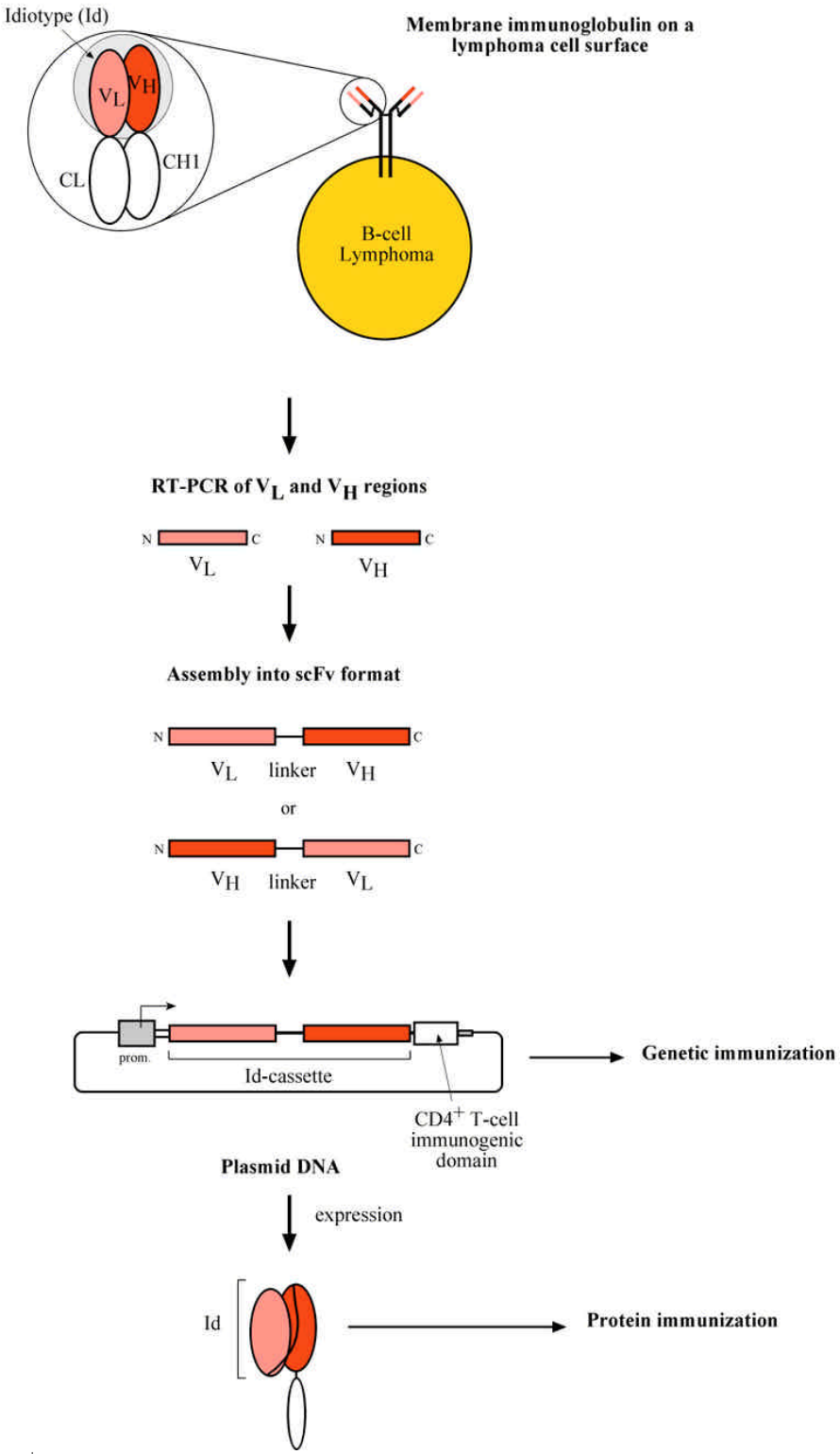

Figure 1. From surface Ig^+ lymphoma cells to scFv vaccines. The scFv format allows to obtain in a single molecule the same relative orientation of the two structurally relevant Id domains, V_L and V_H, of the membrane Ig expressed by the lymphoma. An immunogenic carrier protein is needed to provide T helper cell epitopes. Either the Id-protein or the plasmid encoding it can be used to induce anti-Id responses.

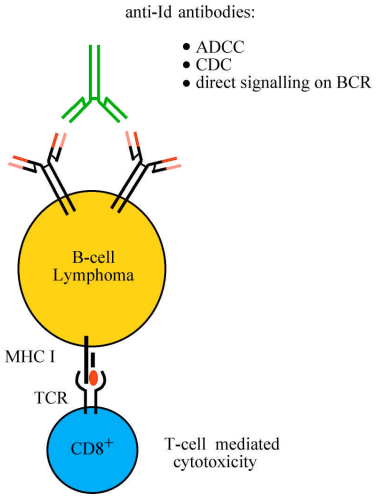

Figure 2. Different arms of the immune system could be active in tumor rejection. Anti-Id antibodies could activate antibody dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC) or induce direct inhibition of tumor growth by anti-idiotypic signaling through the surface immunoglobulin. Cellular immunity by idiotype-specific $CD8^+$ T cells would recognize MHC class I/peptide complexes on the surface of lymphoma cells.

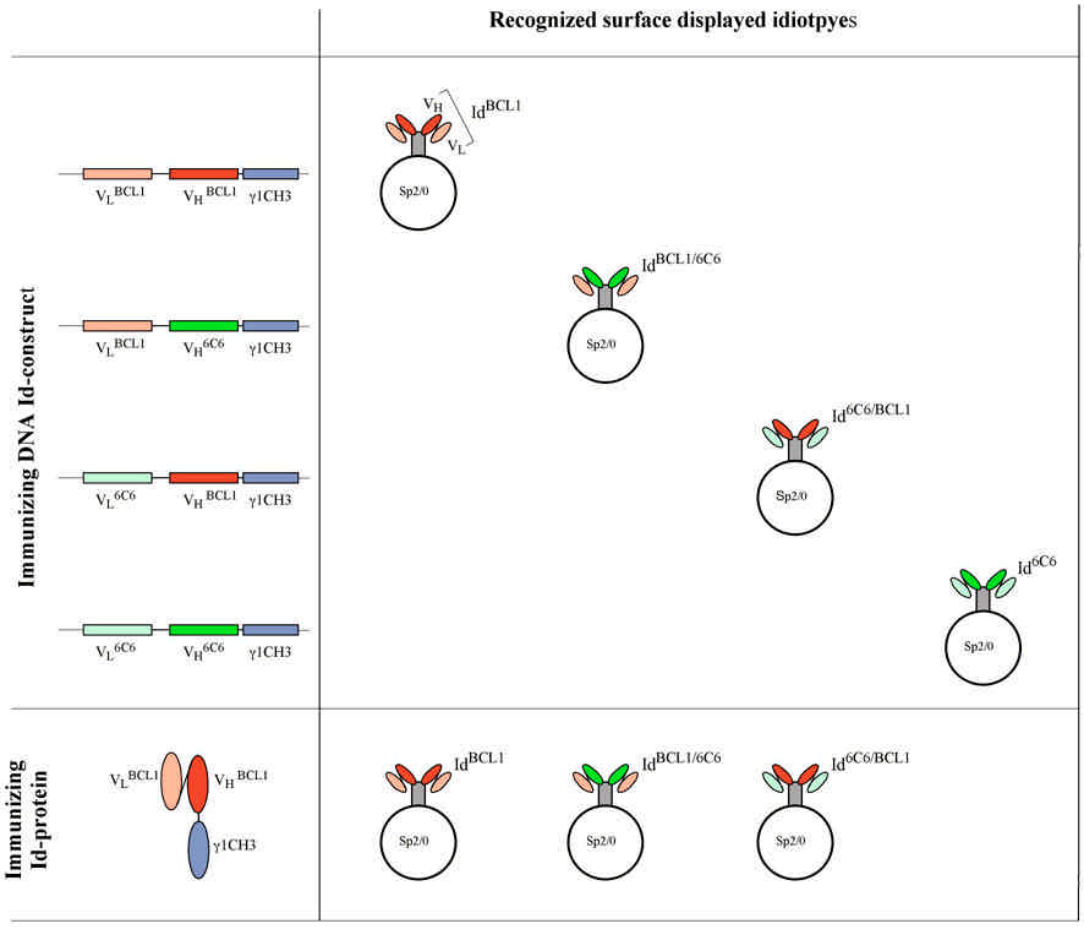

Figure 3. Specificity of anti-Id antibodies. The specificity of the anti-Id antibodies depends on the immunogen used: naked DNA immunizations induce responses recognizing only determinants of the combined V_L/V_H, while protein immunization induces antibodies recognizing also Ids containing only one of the two V regions. The analysis was performed in Sp2/0 cells transfected with membrane displayed idiotypes (21) to guarantee proper folding.

Interestingly, the same immunogen delivered as scFv protein produced in transfected mammalian cells, induced antibodies directed also against single V region (V_L and V_H) specific determinants (figure 3) (21). This may reflect the different availability and processing of antigenic peptides when the antigen is produced *in vivo*, as in the case of DNA immunization, or expressed and manipulated *ex-vivo*, as in the case of protein immunization. It remains to be elucidated whether these observations have a direct implication on vaccine efficacy, but it is interesting to note that DNA vaccination was shown to be as efficient as protein immunization in inducing protection, although total levels of anti-Id antibodies against the lymphoma immunoglobulin were much lower (20).

5. CONCLUSIONS

A serious limitation to design vaccines against cancer is the scarce availability of defined tumor specific antigens. Idiotypes expressed on the surface of B-lymphoma cells offer an ideal model to define the requirements to activate the immune system against self-antigens. Vaccination using idiotypic protein has provided encouraging results in clinical trials, demonstrating the possibility to activate the immune system against idiotype derived epitopes. However, the production of patient specific idiotypic protein is a time consuming procedure that limits large-scale application. Cloning of immunoglobulin genes to be delivered directly as plasmid DNA is an appealing alternative that has been validated by several studies in mouse models. Moreover, the ease to manipulate antigen-encoding cassette has facilitated the analysis of different vaccination strategies and the requirements to obtain high and specific immune responses. Several stratagems to induce protective responses have already been exploited and more will develop as the knowledge of the mechanisms involved in the induction and regulation of immune responses increases. While waiting the results of the first clinical trials of genetic vaccination in humans, mouse models remain a valuable system to analyze new formulations and to understand the immunological mechanism of tumor protection.

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