Somatic transgenesis by immunoglobulin genes

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Summary

In this chapter we describe and discuss somatic transgenesis produced in adult immunocompetent mice using plasmid DNA containing immunoglobulin genes under control of tissue-specific regulatory elements. We review our experience to date and discuss the findings in relation to the known rules for intracellular usage of immunoglobulin genes in activated and differentiated B cells. Because immunoglobulin genes are controlled by B lymphocytes specific promoter and enhancer elements, somatic transgenesis is a new approach to selective targeting of B lymphocytes *in vivo* for transcription and long-term expression of exogenous immunoglobulin genes. Owing to the fact that transgenic immunoglobulins synthesized and secreted *in vivo* are immunogenic for the host and that immunoglobulin genes can be engineered to code for heterologous epitopes, ligands or receptors, somatic transgenesis offers unique features for the development of new strategies of DNA-based immunization and gene therapy.

I. Introduction

Advances in molecular medicine are based on the possibility to efficiently deliver genes into specialized tissues. Two essentially independent disciplines are predicated on this technological approach, gene therapy (Mulligan, 1993) and DNA vaccination (Cohen, 1993; Donnelly et al., 1997). In both instances success is determined by a series of factors all of which depend on the efficiency of gene delivery and gene expression *in vivo*. Strategies have been developed to realize gene delivery via receptor mediated pathways (Ferkol et al., 1995; Ferkol et al., 1996; Wu et al., 1989) exploiting specific structures on somatic cells and their mechanisms to internalize and transport macromolecules. Targeted delivery of DNA also needs to be gauged through the specificity of regulatory elements, i.e., promoters and enhancers, which allow the transgene to be transcribed and translated only in selected tissues. The efficiency of these processes constitutes the rate limiting factor for *in vivo* efficacy of both gene therapy and DNA vaccination.

The use of polynucleic acid for vaccination is complicated by the necessity to achieve sufficient secretion of the transgene product in an immunogenic form. An ideal DNA vaccine should yield molecules with sufficient conformational resemblance to the native antigen to elicit immunity with the greatest cross-reactive potential. In addition, the process of DNA vaccination should also be tailored to include in the process the participation of antigen-presenting cells to heighten both humoral and cellular immune responses.

It is based on this reasoning that we developed a new, rational method of immunization using DNA molecules, somatic transgene immunization (STI), to combine selective targeting of B lymphocytes and their antigen-presenting capacity with expression of conformationally constrained antigen molecules (Gerloni et al., 1997). We directed our considerations to immunoglobulin (Ig) genes as "immunogens" in DNA vaccination. In this chapter we will review what known about the principles of immunogenicity by DNA and subsequently describe our findings on somatic transgenesis as they relate to *in vivo* targeting of B lymphocytes.

II. DNA and immunogenicity

DNA itself is scarcely immunogenic as it has proven extremely difficult to induce a response against DNA (Madaio et al., 1984) even though there exist clinical situations in which autoantibodies to double and single stranded DNA are produced (Koffler et al., 1969; Pincus et al., 1969; Tan et al., 1966). The immunogenicity of DNA *per se* depends on its origin, *i.e.*, eukaryotic or prokaryotic. For instance it was shown that mice

immunized with *Escherichia coli* DNA complexed with methylated BSA in adjuvant produce significantly greater amounts of antibodies than mice similarly immunized with calf thymus DNA (Gilkeson et al., 1989; Gilkeson et al., 1989). This indicated that DNA molecules differ in their immunogenic potential, a characteristic likely due to unique sequences or structures present in bacterial DNA but rarely in mammalian DNA (Gilkeson et al., 1989; Gilkeson et al., 1989). Bacterial DNA possess immunostimulatory properties (Tokunaga et al., 1984), is mitogenic for B cells and induces polyclonal antibody production (Messina et al., 1993), and enhances the lytic activity of natural killer cells with production of IFN- (Tokunaga et al., 1984; Yamamoto et al., 1992). This stimulatory properties are linked to a six-base nucleotide motif consisting of an unmethylated CpG dinucleotide (Krieg et al., 1995) expressed nearly twenty times more frequently in bacterial than in vertebrate DNA (Cardon et al., 1994).

In 1992 Tang and coworkers reported that inoculation of plasmid DNA induces specific immunity *in vivo* (Tang et al., 1992). There is a fundamental difference between Tang's accomplishment and previous attempts to immunize against DNA as it showed that it was possible to use functional genes to generate immunity against a specific gene product. Implicit in this discovery was the fact that factors regulating gene expression would also regulate immunogenicity *in vivo*. Many reports have appeared since demonstrating the use of plasmid DNA in eliciting immunity against viruses (Davis et al., 1993; Raz et al., 1994; Ulmer et al., 1993; Wang et al., 1993), bacteria (Huygen et al., 1996; Tascon et al., 1996), parasites (Doolan et al., 1996; Sedegah et al., 1994; Xu and Liew, 1995), tumor antigens (Conry et al., 1994), self antigens (Gilkeson et al., 1996; Waisman et al., 1996) and allergens (Hsu et al., 1996; Raz et al., 1996). The rapidly raising hope to develop simple and cost effective methods of vaccination was followed by the demonstration that plasmid DNA could be used to induce antibody responses (Cox et al., 1993; Davis et al., 1994; Fynan et al., 1993; Raz et al., 1994; Robinson et al., 1993; Sedegah et al., 1994; Ulmer et al., 1993; Wang et al., 1993) as well as cell-mediated responses of helper (Xiang et al., 1994) or cytotoxic T-cell type (Raz et al., 1994; Sedegah et al., 1994; Ulmer et al., 1993; Wang et al., 1993).

III. The development of somatic transgene immunization

Immunization via DNA inoculation relies on *in vivo* transfection, production and possibly secretion of the transgene product, and antigen presentation by specialized cells. However, in most studies neither the *in vivo* transfected cells nor the antigen presenting cells involved in the process have been identified. Most experiments use foreign DNA under the control of viral promoters which have limited tissue specificity. Therefore, no tissue-specific control of expression is possible other than the site of DNA inoculation.

Since the quality of nucleic acids and gene expression are intimately connected with the vaccination process we began studies considering together three factors that, alone or in combination, could affect the success of DNA immunization: (**i**) the efficiency of *in vivo* transfection including DNA uptake by the host cells, (**ii**) the efficiency with which transfected cells can utilize the DNA and synthesize the transgene product, and (**iii**) the ability of *in vivo* transfected cells to serve as antigen-presenting cells (APCs). Most reports still indicate that expression of the transgene, synthesis of the corresponding gene product, its presentation to immunocompetent cells and induction of immunity occur after repeated inoculations of plasmid DNA (Whalen and Davis, 1995).

The genetic organization and the molecular events leading to expression of immunoglobulin genes are well, albeit not completely, understood and consist in a cascade of tissue-specific genetic events occurring during B-cell differentiation and resulting in the synthesis of immunoglobulin molecules (Alt et al., 1987; Sleckman et al., 1996). Studies *in vitro* have shown that transfection of B cell lymphomas with rearranged Ig gene is followed by a prompt utilization of the transgene and by secretion of immunoglobulins encoded by the transgene (Morrison, 1985). A variety of rearranged functional Ig genes have also been introduced in the germline to create mice with homogeneous antigen receptor expression on B cells and secretion of immunoglobulins with identical chemical and immunologic characteristics (Storb, 1987). It is then obvious that DNA immunization with Ig genes, *e.g.*, heavy (H) chain genes under the control of their own tissue-specific promoter and enhancer elements (Banerji et al., 1983; Gillies et al., 1983; Grosschedl and Baltimore, 1985; Mason et al., 1985) could be a way to target B lymphocytes and satisfy basic requirement for immunogenicity such as synthesis and secretion of the transgene product, and antigen presentation.

In our first experiments we inoculated adult C57Bl/6 mice with a plasmid DNA containing a chimeric (mouse/human) immunoglobulin H chain gene with tissue-specific promoter and enhancer elements. We immediately observed that a single intraspleen inoculation was followed by (*i*) uptake and persistence of the transgene in B lymphocytes for 3-4 months, (*ii*) secretion of transgenic immunoglobulins (transgene H chain + endogenous L chain) in amounts ranging between 15 and 30 ng/ml, and (*iii*) production of IgM anti-Ig antibodies. The circulating H chain transgene product was found to be associated prevalently with light (L) chains a fact per se not surprising since L chains represent 95% of the pool of genes utilized *in vivo* in the mouse. A booster immunization with Ig coded by the same transgene at various times after priming with DNA showed the generation of a typical secondary immune response with IgG1 and IgG2b antibodies (Gerloni et al., 1997). Thus, a single inoculation of an Ig H chain gene targeted to spleen lymphocytes was sufficient to initiate immunity

and establish immunologic memory. We termed this process somatic transgene immunization (STI) to reflect the fact that a foreign gene is transported inside somatic cells and initiates immunity (Gerloni et al., 1997). It became evident that from this point on the physiological machinery provided by STI could be exploited to program one's individual immune response in a rational way.

IV. Investigations on the transgene

Intraspleen inoculation of the H chain transgene resulted in immunity and established immunologic memory. To explore the basis for this phenomenon we undertook a systematic analysis of (**i**) the tissue
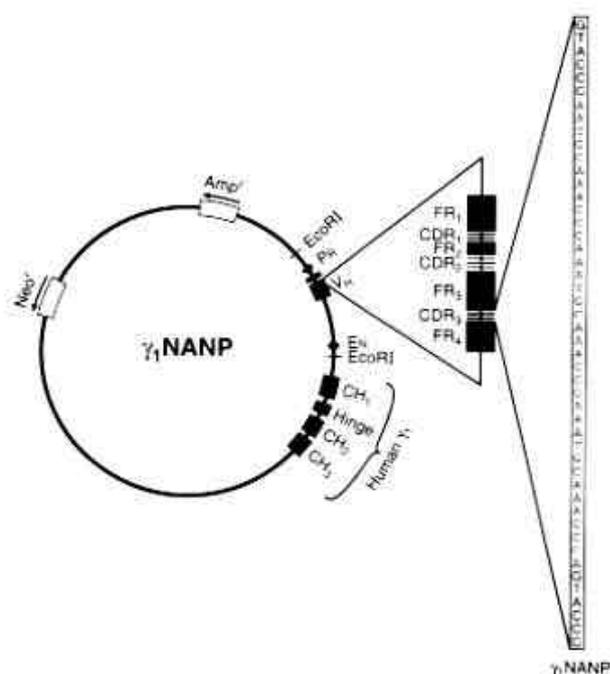
Figure 1. Schematic representation of plasmid 1NANP. The plasmid contains the Ig H chain construction which is the product of the fusion between the productively rearranged murine V_H^{62} gene and the human 1 C region gene present in genomic configuration in plasmid vector pNeo 1 (Sollazzo et al., 1989). The VH gene was modified by insertion in the CDR3 of 36 bp heterologous sequence coding for three repeats of the amino acid sequence Asn-Ala-Asn-Pro (NANP). The plasmid DNA carries the regulatory elements, promoter (Pr) and enhancer (En) needed for tissue-specific expression. Neo[r] = neomycin and Amp[r] = ampicillin, are the resistance genes. CDR = complementarity-determining region. FR = framework region.

distribution of the transgene, (**ii**) its fate *in vivo* over time, (**iii**) the cell type involved in targeting, and (**iv**) the potential for somatic mutation of the transgene *in vivo*.

These studies were based on an Ig H chain gene purposely engineered in the CDR3 of the VH domain to have a 36 base-pair exogenic molecular marker (**Figure 1**).

A. Tissue distribution

Genomic DNA extracted from various lymphoid (*i.e.*, spleen, lymph-nodes and bone-marrow) and non-lymphoid (*i.e.*, liver, kidney, lung and muscle) tissues explanted at different times, was analyzed for specific amplification of the transgene VDJ by PCR and Southern blot hybridization. An amplification product was readily visible in splenic genomic DNA. No specific amplification occurred in any of the other tissues (**Table 1**). This did not vary at any of the time points analyzed. To control for specificity and increase the sensitivity of the reaction, two additional PCR assays were performed using primers designed to anneal sites within the VDJ region. One set of primers (pSE/pNAD) specifically amplified the molecular maker; another (inner primers: pNEL/pNED) served for nested PCR (**Figure 2**). The results confirmed those obtained with VDJ amplification. Southern blot analysis using a probe specific for the molecular marker further confirmed the PCR results (**Table 1**). Thus, after intraspleen inoculation the transgene H chain persists *in vivo* for a period of 3 months in the organ in which it was inoculated.

B. Fate of the transgene

PCR and Southern blot hybridization were also used to monitor the kinetics of the presence of the transgene *in vivo* in mice analyzed at various times after DNA inoculation (Xiong et al., 1997). Amplification of the transgene VDJ region was visible up to 12 weeks after a single DNA inoculation. No amplification was seen at subsequent time points (16, 24, 36 and 52 weeks) (**Table 1**). Southern blot hybridization with the marker-specific probe further confirmed the PCR results (**Table 1**).

C. The transgene is harbored in B lymphocytes

As discussed above mice undergoing STI produce transgene Ig (15-30 ng/ml) for a protracted period of time *in vivo*. Coupled with the demonstration that the transgene could only be found in the spleen (the organ of inoculation) and consistent with the use of a gene under control of promoter and enhancer elements specific for B lymphoid cells, it became obvious that B lymphocytes could be the cell population accounting for the initiation of STI (*i.e.*, transgene uptake, the persistence of the transgene, its transcription and secretion of transgenic Ig).
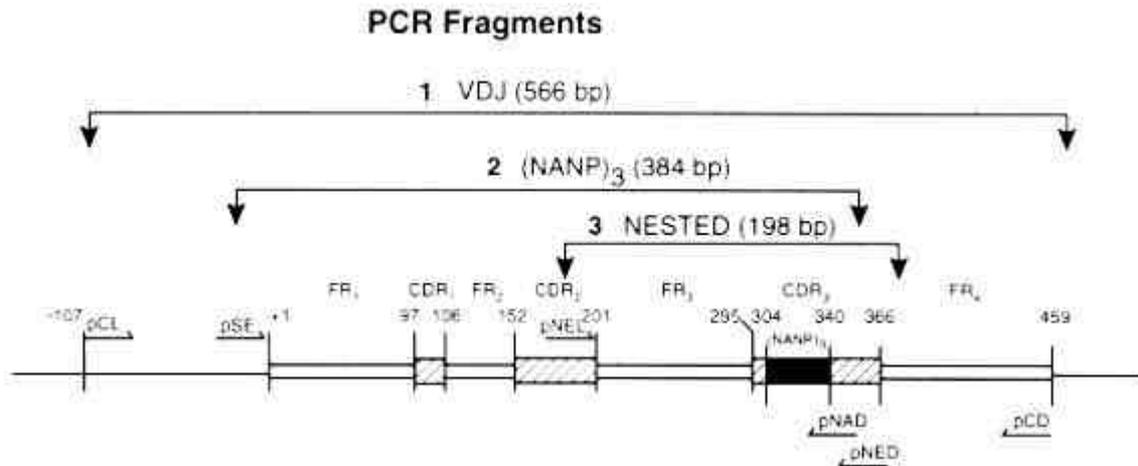
PCR Fragments

Figure 2. Schematic representation of the V_H gene contained in plasmid 1NANP DNA. The annealing sites of the primers, the predicted amplification fragments and their molecular size, are identified. VDJ refers to a fragment inclusive of the coding region for the rearranged V-D-J gene segments; (NANP)3 refers to a 384 bp fragment containing the coding sequence for three NANP repeats in the CDR3 of the V_H region between nucleotides 304-340; NESTED refers to a 198 bp fragment inclusive of the coding region for FR3 and the CDR3. Any other position in the gene is numbered in reference to nucleotide +1 (the first nucleotide in the coding region of FR1).

Table 1. PCR amplification and Southern blot hybridization of the transgene *in vivo*

Time (weeks)	Spleen	Lymph nodes	Bone Marrow	Liver	Kidney	Lung	Muscle
0*	-	-	-	-	-	-	-
4	+/+	-	-	-	-	-	-
12	+/+	-	-	-	-	-	-
16	-	-	-	-	-	-	-
24	-						
52	-						

* Time zero refers to results generated with tissues extracted from a naive mouse (negative control). PCR amplification of the murine -actin gene served as an internal control.
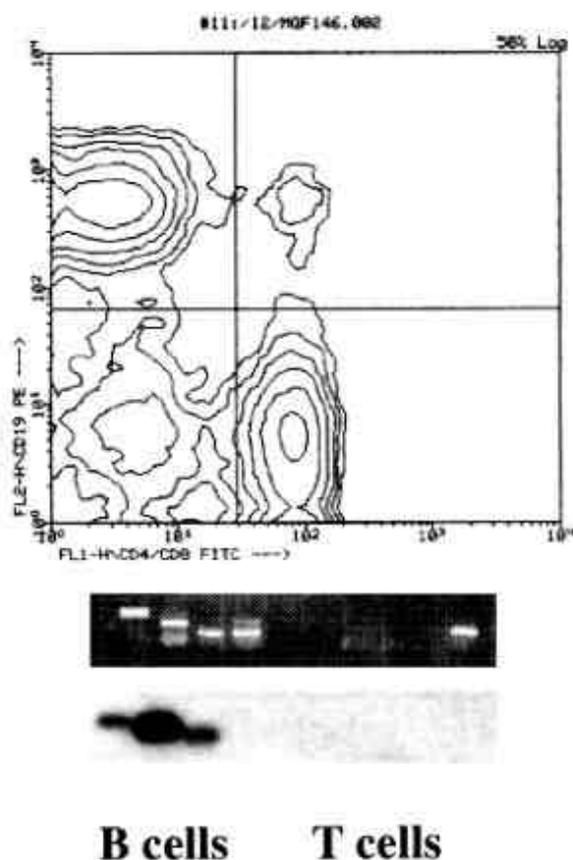Plus signs correspond to positive PCR amplification (+/) and positive Southern blot hybridization (/+)

To formally demonstrate this   assumption we analyzed populations of spleen B and T cells (Xiong et al., 1997). Splenic B and T lymphocytes were isolated to a high degree of purity (97-99%) by FACS sorting and their respective genomic DNA was amplified by PCR. Twenty-eight days after DNA inoculation distinct amplification products were detected in B lymphocytes (**Figure 3**). Southern blot hybridization confirmed the specificity of the amplification products. It appears, therefore, that B lymphocytes in the spleen are the cell population targeted by the Ig H chain gene. The transgene could not be amplified from peripheral blood lymphocytes.

Questions yet to be answered are "How is the transgene internalized in B cells?" and "How many B cells are part of this process *in vivo*?" As to the first question several possibilities are considered such as uptake of plasmid DNA by B cells may have occurred either through surface Ig specific for DNA (Glotz et al., 1988; Holmberg et al., 1986) or through the non-Ig receptor for DNA described in murine and human lymphocytes (Bennett et al., 1985). As to the second question we have no definitive answer yet. Suffice it to say that the phenomenon of STI is supported by a limited number of B cells transfected *in vivo*. We estimated these cells to be fewer than 5×10^4/spleen or 0.07% of total cells.
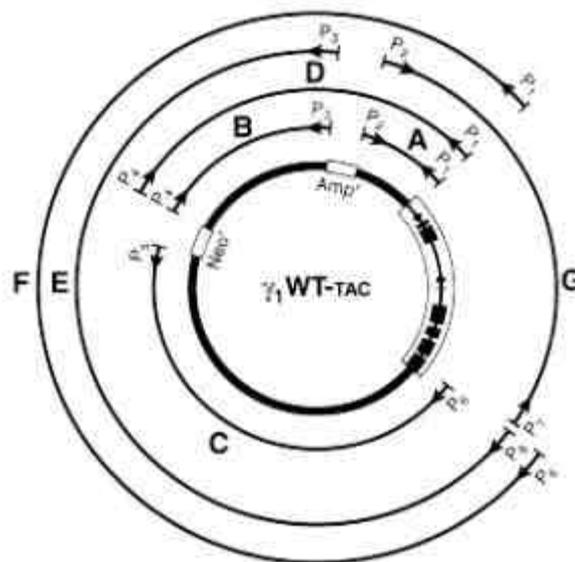
D. Integration in the host genome

Protracted secretion of transgenic Ig led us to consider the possibility of integration of the H chain transgene in the host genome. Two approaches were pursued. In the first one (**Figure 4**), genomic DNA extracted from splenic tissue harvested 17 days after DNA inoculation was analyzed using a multiplex PCR approach (Chen et al., 1994; Daniel et al., 1995; Donaldson et al., 1993) with ELONGASE, a mix of DNA polymerases with improved proofreading activity for amplification of large (<20 Kb) DNA fragments, and seven pairs of specific primers to amplify seven different fragments of plasmid 1WT DNA. Primers were designed to facilitate the detection of

fragmented plasmid and integration break-points in the plasmid, if they occurred (*i.e.*, failure to amplify one or several DNA segments in the genomic DNA would demonstrate integration). In experiments repeated under a wide range of $MgCl_2$ molar concentrations as well as different annealing temperatures two fragments consistently failed to amplify. This pattern indicated a break-point localized in or around the neomycin resistance gene (Gerloni et al., 1997). In the second approach (**Figure 5**) genomic DNA from splenic tissue was first digested with Xba I and subsequently ligated and amplified (Xiong et al., 1997). We reasoned that a pattern of multiple molecular size products (smear) would suggest integration of the transgene whereas a single band of m.w. ~ 15 kb would suggest persistence of the transgene in episomal form. Both genomic DNA extracted from the spleen 4 and 12 weeks after plasmid DNA inoculation gave rise to amplified products of multiple molecular sizes (a smear). No such a pattern was observed in the genomic DNA extracted 36 weeks after inoculation, consistent with the kinetics of transgene detection. The presence of a non-integrated (episomal) form of the transgene (in addition to the integrated one) was sought by PCR amplification in which again an ELONGASE mix was used to ensure amplification of large (20 kb) DNA fragments. We reasoned that if plasmid DNA exists in episomal form, a

Figure 3. Isolation of splenic B and T lymphocytes and detection of the H chain transgene in the purified lymphocyte populations. B and T lymphocytes from the spleen of DNA-inoculated mice were sorted and purified on a fluorescence-activated cell sorter (FACS) 28 days after DNA inoculation. From left to right the lanes are as follows: lane 1 - fragment amplified with the primers pCL/pCD (VDJ); lane 2 - fragment amplified with the primers pSE/pNAD [(NANP)3]; lane 3 - fragment amplified with the primers pNEL/pNED (NESTED); lane 4 - fragment amplified with the primers pbA1/pbA2 specific for the murine -actin gene (internal control).
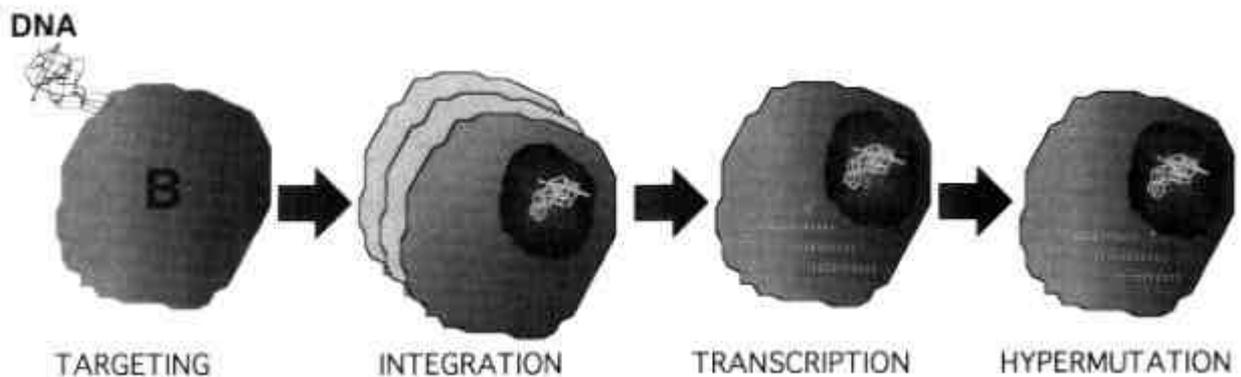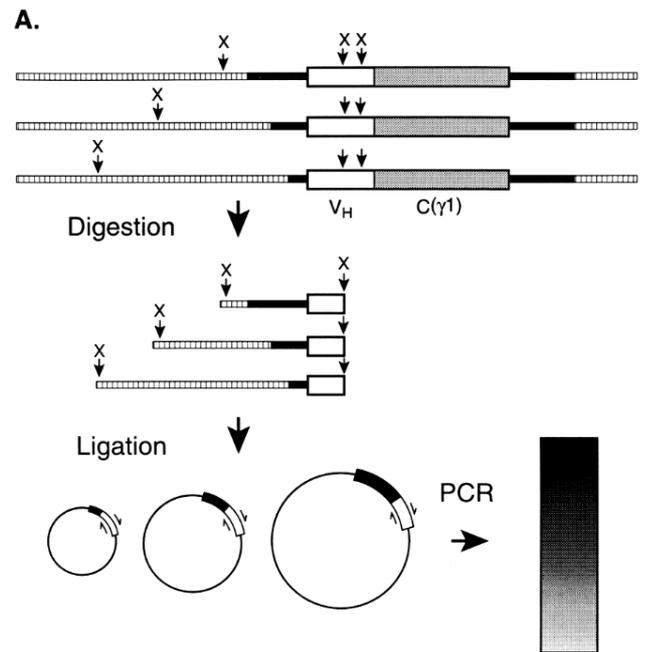

Figure 4. Schematic representation of multiplex PCR analysis to determine integration of the immunoglobulin H chain transgene into chromosomal DNA. Plasmid 1WT-TAC (to scale) and localization of plasmid fragments (A through G) used to analyze integration. P1 through P7 refer to specific primers and their topographical site of annealing. Neo^r = neomycin and Amp^r = ampicillin are the resistance genes. Plasmid 1WT-TAC is in every respect identical to plasmid 1NANP with exception of the NANP-coding sequence which has been deleted.

sharp band of molecular size corresponding to the reference plasmid DNA would be seen. No sharp band corresponding to the reference plasmid DNA was observed. We concluded that only the integrated form is present. By Southern blot the hybridization pattern was similar (smear) to the amplified PCR products.

E. Lack of somatic mutation

The antigenic and immunogenic potential of a transgene-encoded product relies on the fact that no sense somatic mutation will affect the nucleotide sequence of the transgene while this is harbored *in vivo*. Hypermutation occurs frequently in the VDJ region of Ig, mainly in the CDRs, in agreement with the notion that hypermutation takes place during antigen selection and affinity maturation of the antibody response (Griffiths et al., 1984). Although

Figure 5. Schematic representation of the approach and rationale used to demonstrate integration of the transgene. Genomic DNA is digested with Xba I which will cut (**X**) at multiple sites in the chromosomal DNA and in two sites in the transgene H chain. The digested DNA fragments are then re-circularized with T4 DNA ligase and subsequently amplified by PCR using a set of primers (p62L/p62U) designed to anneal and extend in opposite directions (see). If genomic DNA contains the H chain transgene (integration), PCR amplification will give origin to a multitude of DNA products within an extended range of molecular sizes. This will be reflected in a pattern of diffuse gel migration (smear). Symbols are as follows: ( ) = splenic genomic DNA; ( ) = backbone of plasmid DNA g1NANP; (**X**) = Xba I site; V_H = variable region of the transgene H chain; C(g1) = constant region of the IgG1 subclass of the transgene H chain.

Figure 6. Schematic representation of the four main events relevant to somatic transgenesis by immunoglobulin genes.

Table 2. Lack of transgene mutations in PCR-generated clones from splenic genomic DNA

TIME (Weeks)	No. of Clones Sequenced	No. of Clones Mutated	No. of Nucleotides Mutated	Rate of Mutation* (%)
2	6	1/6	1**	2.9×10^{-4}
4	3	0/3	0	-
12	3	0/3	0	-

* Number of mutations /total number of base pairs sequenced.

** A silent (C ->T) mutation in FR3.

the Ig H chain gene used lacks a transmembrane domain, rendering cell surface anchoring unlikely, experiments were performed to assess accumulation of mutations as a result of protracted *in vivo* persistence in integrated form. The transgene VDJ region was amplified from splenic genomic DNA, subcloned and sequenced by the dideoxy termination method. No evidence of hypermutation was found in the VDJ region of the transgene *in vivo* even after 3 months (**Table 2**).

V. Concluding remarks

The studies presented in this chapter indicate that the biological phenomenon of somatic transgenesis relies on series of cellular events which we begin to understand and which seem to fulfill most of the conditions posed as requirements for a new, rational approach to DNA-based immunization. For sake of brevity our comments will concentrate on four points essential in our opinion to understand the phenomenon and the future potential of our work (**Figure 6**).

First, is targeting of B lymphocytes *in vivo*. As mentioned above two main considerations guided our experiments in this direction: One consideration is that B lymphocytes have receptors with specificity for DNA. This makes it possible that DNA, which sticks to the surface of a cell due to its negative charge, is subsequently internalized by receptor-mediated endocytosis. As pointed out both Ig and non-Ig receptors for DNA exist on normal B lymphocytes (Bennett et al., 1985; Glotz et al., 1988; Holmberg et al., 1986). Presumably, after internalization only a fraction of the DNA resists degradation in the endosomes and reaches the cytosol. For instance, 24 hours after *in vivo* receptor mediated gene transfer there exists only 1 copy of DNA per cell compared to 100 copies at four hours (Wilson et al., 1992). To analyze conditions of binding and internalization new experiments are in progress to determine whether the same effect can be obtained *in vitro*. The other consideration is that tissue specificity is provided by the Ig gene regulatory elements (see below).

Second, is integration. Studies in mice transgenic for Ig genes have sufficiently shown that transgenes are generally integrated in multiple tandem copies at one or a few sites in the host genome. Importantly, integration does not occur into the homologous H or L locus (Storb, 1987). Similarly, somatic cell hybridomas and non-secreting B-cell lymphomas transfected with Ig genes both harbor integrated foreign gene(s) (Morrison, 1985) randomly. On the other hand, site specific integration can be achieved using suitably modified expression vectors such as replacement (Kardinal et al., 1996) or integration (Lang and Mocikat, 1994) vectors. From the foregoing, it does not surprise that during somatic transgenesis integration occurs randomly. It is quite likely that the transgene enters the nucleus during cell division (**Figure 6**).

An aspect intimately connected with integration of the transgene and its expression in B cells is its relation with the endogenous Ig gene since, as a rule, a single B cell expresses only one H chain together with one L chain, allelic exclusion (Pernis et al., 1965). Although at this stage we have no data in favor or against allelic exclusion during somatic transgenesis, it is of note that germ-line transgenic mice have variably shown some leakage and that the endogenous Ig gene is expressed together with the transgene product (Storb, 1995). Apart from the cell biological relevance, even partial lack of allelic exclusion in somatic transgenic cells would lead to secretion of mixed antibody molecules. Further studies will need to address this point and their potential implication for immunization.

Third, is the process of transcription and translation. Possibly this is the step that confers the highest degree of selectivity to the entire process. It is well known that Ig gene expression is restricted to lymphoid cells and among them to cells of the B-cell lineage (Banerji et al., 1983; Gillies et al., 1983; Grosschedl and Baltimore, 1985; Mason et al., 1985). Therefore, the use of B cell specific promoter and enhancer elements introduces a stringent control mechanism on tissue specificity and utilization of the transgene *in vivo*. One essential aspect to a full understanding of the events starting somatic transgenesis is

"How does the first B cell become activated and how does this B cell begin to produce transgenic Ig molecules?" A plausible explanation is that bacterial DNA of the plasmid backbone possesses immunostimulatory properties for B cells (Messina et al., 1993; Tokunaga et al., 1984), an activity mediated by a six-base, unmethylated CpG dinucleotide (Krieg et al., 1995). Thus, activation of B cells by plasmid DNA may be crucial to initiate transcription and translation, and ultimately to set in motion the immunogenic process.

Fourth, is somatic mutation. It was appreciated early on that Ig genes isolated from myelomas and hybridomas are mutated in the V region compared with the corresponding germ-line (Crews et al., 1981). Hypermutation occurs frequently in the VDJ region, mainly in the CDRs. This is commonly explained with hypermutation taking place during antigen selection and affinity maturation of the antibody response, and is an important means to increase antibody diversity (Griffiths et al., 1984). Hypermutation arises through one of two mechanisms: antigen selection or intrinsic mutational bias independent of selection. In the first case it is required that the Ig is expressed at the surface of B lymphocytes for antigen to exert selective pressure. As indicated in **Figure 1** the transgene used in our studies lacks a transmembrane domain. This renders cell surface anchoring unlikely with no possibility for somatic mutation to occur via this mechanism. Another consideration is that we used a H chain transgene coding for an already rearranged V region segment, hence ruling out the possibility of mutations introduced during rearrangement. Others have shown that transgenic mice engineered with already rearranged chain genes do not mutate unless hyperimmunization is in place (O'Brien et al., 1987). In the second case, *i.e.*, transcriptional error or transcription-driven hypermutation, there appears to be a dependence on the physical distance between the exon and the promoter element (Peters and Storb, 1996). This does not seem to apply to our model of somatic transgenesis as no mutation was found among the clones examined, albeit only a limited number of clones at selected time points were sequenced (**Table 2**). Understanding lack of transcription-driven hypermutation during somatic transgenesis will need to be addressed with further studies.

In conclusion, we have shown and discussed the use of H chain Ig genes in somatic transgenesis as an *in vivo* step of targeted transgene expression which preceding the phase of immunogenicity which is central to our attempts to develop a new rational approach to immunization, somatic transgene immunization. Others have used a similar rationale to target B lymphocytes *in vivo* using retroviral vectors for stable transgene expression (Sutkowski et al., 1994). The considerations made in this paper are relevant to better understand the nature of somatic transgenesis and to its future applications for DNA vaccination and gene therapy.

Acknowledgments

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