

***In vivo* production of therapeutic antibodies by engineered cells for immunotherapy of cancer and viral diseases**

Review Article

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Summary

Our recently developed ability to produce human monoclonal antibodies, together with that of reshaping antibody molecules, offers new tools for treating a number of human diseases. Direct injection of purified antibodies, or of antibody-related molecules, to patients would, however, not always be possible or desirable. This is especially true in the case of long-term therapies for at least two reasons. One is the high cost of antibodies certified for human use. The other is the possibility of neutralizing anti-idiotypic immune responses as a result of repeated injection of massive doses of antibody. *In vivo* production of therapeutic antibodies through either genetic modification of patients' cells or implantation of antibody-producing cells might overcome both of these hurdles. Several cell types suitable for use in cell/gene therapy protocols, such as skin fibroblasts, keratinocytes, myogenic cells and hepatocytes, are capable of producing monoclonal antibodies *in vitro* upon gene transfer. Furthermore, the grafting of engineered myogenic cells permits the long-term systemic delivery of recombinant antibodies in immunocompetent mice. Importantly, antibodies produced both *in vitro* and *in vivo*, retain the specificity and the affinity of the parental antibody and no anti-idiotypic response is detected in mice producing ectopic antibodies. Long-term systemic delivery of such antibodies into mice can also be achieved through the implantation of antibody-producing cells encapsulated into a new biocompatible material, cellulose sulphate. Importantly, no inflammation occurs at capsule implantation sites over periods as long as 10 months. Moreover, no anti-idiotypic response develops against antibodies released by encapsulated cells. Encapsulation of antibody-producing cells in immunoprotective devices should offer multiple advantages over genetic modification of patients' cells. These include protection against immune cells of treated individuals, the possibility of easy removal of implanted cells as well as that of implantation of non-autologous cells. Taken together, these observations demonstrate that long-term *in vivo* production and systemic delivery of monoclonal antibodies is technically feasible. Application of this technology to the treatment of various viral and autoimmune diseases as well as that of cancer is currently underway.

I. Introduction

Specific antibodies can be generated against virtually any type of molecule since antigens can be proteins, nucleic acids, lipids or glucids. They can also be self or

foreign. The potential of clinical applications for antibodies is thus enormous and concerns a wide range of diseases including cancer, viral infections, transplant rejection, autoimmunity, toxic shock, rheumatoid arthritis, and restenosis (Chester and Hawkins, 1995).

Since the discovery of monoclonal antibodies in 1975, various antibody-based therapies have been tested, mostly for treating patients suffering from cancer. However, the poor efficiency of the first monoclonal antibodies used in clinical trials, the development of neutralizing immune responses by patients against antibodies of animal origin and the long periods of time necessary for forming a proper view of the efficacy of treatments have momentarily tempered the initial enthusiasm raised by this technology. Nevertheless, the therapeutic successes obtained during the past years (Scott and Welt, 1997) and the rapid developments of antibody engineering have brought therapeutic monoclonal antibodies back to the fore. Among the therapeutic successes, one can mention a variety of anti-idiotypic antibodies for treating B lymphoma (White et al., 1996) and the now commercially available chimeric antibody ICED-C2B8, which is more efficient than conventional chemotherapy for treating non-Hodgkin's lymphomas (Maloney et al., 1997; Marwick, 1997).

The main initial drawback met when administering monoclonal antibodies in human patients, namely the immunogenicity of murine antibodies, can now be overcome following several approaches (**Figure 1**). These include : **(i)** the humanization of animal antibodies using site-directed mutagenesis possibly assisted by computerized molecular modeling (Wawrzynczak, 1995); **(ii)** the generation of hybridomas from transgenic mice harboring the human immunoglobulins loci substituted for the mouse loci (Bruggemann and Taussig, 1997; Mendez et al., 1997); **(iii)** the construction of hybridomas from activated human B lymphocytes (Wawrzynczak, 1995); and

(iv) the screening of bacteriophage libraries expressing human immunoglobulins at their surface (Marks and Marks, 1996; Rader and Barbas, 1997). In addition, gene engineering now allows both the improvement of intrinsic properties of antibodies, such as affinity and avidity, the grafting of new effector or enzymatic functions as well as the construction of new antibody-based molecules such as single chain Fv, bispecific antibodies (Chester and Hawkins, 1995; Wawrzynczak, 1995). In conclusion, molecular engineering of antibodies, together with the possibility of generating human monoclonal antibodies, provide us with new antibodies and antibody-related molecules which will, undoubtedly, find clinical therapeutical applications, especially in the field of gene therapy (Pelegrin et al., 1998).

II. A gene/cell therapy approach for the systemic delivery of therapeutic antibodies.

In theory, the simplest mode of administration of therapeutic antibodies consists of repeated intravenous injection. However, the high cost of antibodies produced under gmp (good manufacturing practice) conditions makes most monoclonal antibodies uneconomic for long-term treatments (several months to several years) on a large scale since numerous antibody-based therapies would involve several tens to several hundreds of mg of antibody per month and per patient. Therefore, clinical application of therapeutic antibodies in the long-term is limited by the necessity of finding financially acceptable delivery systems.

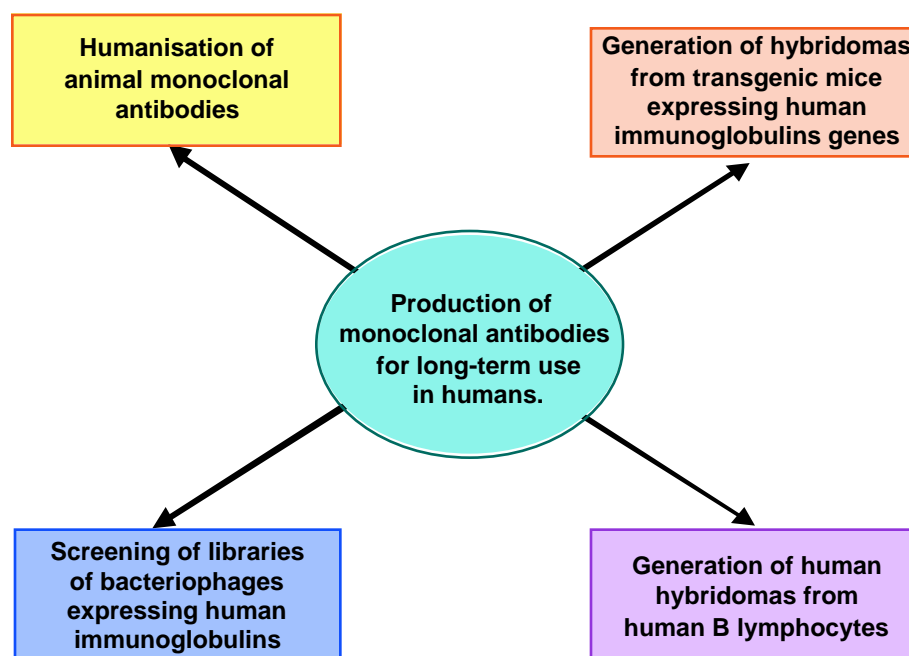


Figure 1. Generation of monoclonal antibodies suitable for long-term use in humans.

To solve this issue, a new gene/cell therapy based on the *in vivo* production of ectopic antibodies through either the genetic modification of patients' cells or the implantation of antibody-producing cells encapsulated within immunoprotective devices are currently being developed in the laboratory. These delivery systems should not only render long-term therapeutic antibody treatments cost-effective but should also provide an additional therapeutic benefit. Continuous and sustained delivery of antibodies at a low, but therapeutic, level should permit the suppression, or at least the delay, of neutralizing anti-idiotypic immune responses which often develop when massive doses of purified immunoglobulins are repeatedly injected (see below).

III. Potential applications of the *in vivo* production of ectopic antibodies.

A first and obvious clinical target for therapeutic antibodies produced *in vivo* is cancer. Long-term production of ectopic antibodies could, thus, be used in the context of surveillance treatments for preventing relapse after a primary treatment consisting of surgery, chemo- or radiotherapy. Providing the basis for future protocols, several antibodies, cytostatic or cytotoxic for tumor cells, have already been characterised (Old, 1995; Riethmüller et al., 1993; Scott and Welt, 1997; Vitetta and Uhr, 1994). Some of them have even been used with success in various clinical trials based on passive administration of purified immunoglobulins (**Table 1**) (Scott and Welt, 1997).

A second target is life-threatening viral diseases, such as AIDS, for which no satisfactory treatment is available to date. The therapeutic antibodies could be virus-neutralizing antibodies, antibodies toxic for virus-producing cells or antibodies specific for cell surface molecules required for viral infection. Supporting the notion that such treatments can be efficiently applied to the curing of viral diseases, transgenic mice expressing a neutralizing antibody are protected from lethal infection by the lymphocytic choriomeningitis virus (Seiler et al., 1998). Also supporting the view of the potential utility of such treatments, it was recently shown that blocking the entry of HIV into target cells by administration of a short peptide (T20) can provide potent inhibition of HIV replication in patients suffering from AIDS (Kilby et al., 1998). In addition, several monoclonal antibodies with a neutralizing effect on HIV, including primary virus isolates, are already available and might be used for passive immunoprophylaxis of AIDS in the future (**Table 2**) (Burton, 1997; Burton and Montefiori, 1997). These antibodies are directed against the envelope glycoprotein subunits, gp120 and gp41, and have originally been characterised in *in vitro* inhibition assays. Some of them can even synergise for inhibiting HIV (Mascola et al., 1997) and SHIV (Li et al., 1998) replication. Finally, some of these antibodies are able to inhibit HIV replication in SCID mice grafted with human peripheral

blood lymphocytes (Burton et al., 1994; Gauduin et al., 1997).

A third therapeutic application would be the treatment of certain chronic inflammatory diseases such as rheumatoid arthritis. *In vitro* experiments and recent clinical data have shown that TNF- is a critical inflammatory mediator of this autoimmune disease and might therefore represent a molecular target for specific immunotherapy (Maini et al., 1995). Indeed, it has been shown that administration of anti-TNF- monoclonal antibodies causes an improvement in the health of rheumatoid arthritis-suffering patients, thus providing evidence that such antibodies might represent efficacious drugs for long-term treatments of this disease (Elliott et al., 1994; Maini et al., 1995). However, the elevated doses necessary for obtaining therapeutic effects as well as their high cost still restrict the use of these antibodies on a large scale.

Besides therapy, *in vivo* production of monoclonal antibodies may also have applications in the laboratory. Although the construction of transgenic mice could, most often, be envisaged to reach the same goal, genetic modification of somatic cells of animals or implantation of antibody-producing cells are expected to represent more versatile and less time-consuming techniques, especially when production of combinations of antibodies is desired. A first example of this application would be the development of new animal models of human autoimmune diseases in which the humoral immune response is responsible for, or contributes to, the development of the pathology (Rose and Bona, 1993). Another interesting application would be continuous cell type-specific ablation for studying the biological role of certain cell lineages in living animals. According to this approach, cytotoxic antibodies recognizing specific cell surface markers would be delivered continuously into the bloodstream of living animals where they could kill cells immediately upon appearance (for example, after a differentiation step) of the cognate antigen at their surface. A third application, called "phenotypic knock-out", could be the systemic delivery of antibodies neutralizing the activity of circulating antigens. Demonstrating the relevance of this approach, expression in the central nervous system of transgenic mice of a monoclonal antibody directed against substance P was able to inhibit the activity of this neuropeptide and was shown to be useful for studying the mechanisms of action of the latter (Piccioli et al., 1995).

IV. *In vivo* production of antibodies by genetically modified cells.

Plasmocytes are the terminally differentiated cells of the B lineage which are responsible for the production and the release of antibodies into the bloodstream (Piccioli et al., 1995). Because of their short life-span (several days to few weeks), they cannot be used for long-term gene/cell therapy. Moreover, they already produce an immunoglobu-

Table 1. Antibody and antibody-based molecules used for immunotherapy of cancer. This list is not exhaustive. (°) corresponds to radiolabelled antibodies and (*) corresponds to immunotoxins. Details of clinical trials are to be found in the indicated references.

Agent	Antigen	Disease	Reference
° [¹³¹ I]-anti-B1 (mouse Mab)	CD20	B-cell lymphoma	Kaminsky et al., 1996; Press et al., 1995
° [⁹⁰ Y]-anti-CD20 (mouse Mab)	CD20	B-cell lymphoma	Knox, 1996
° [⁹⁰ Y]-anti-idiotypic (mouse Mab)	Idiotypic	B-cell lymphoma	White et al., 1996
* IgG HD37-dgA [deglycosylated ricin A] (mouse Mab)	CD19	B-cell lymphoma	Stone et al., 1996
* RF84-dgA [deglycosylated ricin A] (mouse Mab)	CD22	B-cell lymphoma	Amlot et al, 1993
IDEC-C2B8 (human-mouse chimeric Mab)	CD20	B-cell lymphoma	Maloney et al. 1997
M195 (mouse humanised Mab)	CD33	Acute Myeloid Leukemia	Caron et al., 1994; Jurcic et al. 1995
° [¹³¹ I]-M195 (mouse humanised Mab)	CD33	Acute Myeloid Leukemia	Jurcic et al., 1995
CAMPATH-1H (humanised Mab)	CDw52	Chronic lymphocytic leukemia	Osterborg et al., 1997
17-1A (mouse Mab)	Epithelial membrane antigen (EMA)	Colorectal carcinoma	Riethmuller et al., 1994
° [¹²⁵ I]-A33 (murine Mab)	A33	Colorectal carcinoma	Welt et al., 1996; Daghighian et al., 1996
Anti-Le ^y B3-liked to <i>Pseudomonas</i> exotoxin (murine Mab)	Le ^y -Antigen	Colorectal carcinoma	Pai, 1996
MFE-23 (scFv antibody)	carcinoembryonic antigen (CEA)	Colorectal carcinoma	Begent et al., 1996
rhuMabHER (humanised Mab)	p185 ^{HER2}	Breast cancer	Baselga et al., 1996
° [¹³¹ I]-cG250 (human-mouse chimeric Mab)	G250	Renal carcinoma	Surfus et al., 1996; Steffens et al., 1997

lin, the expression of which might interfere with the production of the therapeutic antibody.

It has long been known that several eukaryotic cell types such as yeast and certain insect cells in addition to certain mammalian cell lines can produce antibodies upon appropriate genetic modification (for references, see Noël et al., 1997). Interestingly, this observation raised the possibility that a variety of non-plasmocytic cells could be used for production of immunoglobulins. Indeed, we have recently shown that a number of cell types amenable to genetic modification and suitable for graft to humans can secrete antibodies (Noël et al., 1997). These cells include myogenic cells, hepatocytes, keratinocytes and skin

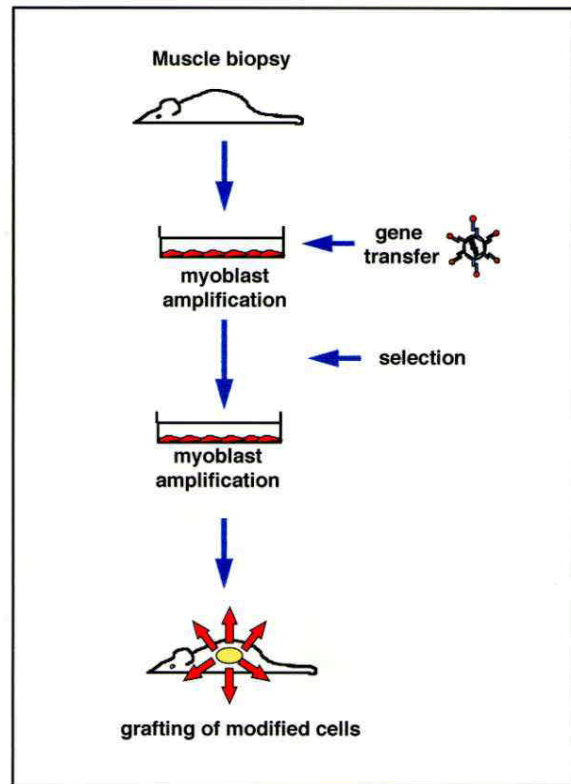
fibroblasts. It is, however, likely that their number will increase in the near future. Furthermore, genetically-modified myogenic cells (Noël et al., 1997) and fibroblasts (unpublished results) grafted to mice were shown to be capable to sustain systemic delivery of cloned antibodies for several months (**Figure 2**). Importantly, the antibodies expressed ectopically *in vitro* and *in vivo* retained the specificity and the kinetic and thermodynamic characteristics of the parental antibody secreted by lymphocytic cells, as assayed using the BIAcore technology (Noël et al., 1997). These data indicate that several (and possibly all) non-B cell types possess the machinery requi-

Table 2. HIV-neutralizing human monoclonal antibodies. Details can be found in the references indicated.

Agent	Antigen	<i>In vitro</i> neutralisation	<i>In vivo</i> neutralisation	References
2F5	gp41 (linear amino acid sequence ELDKWA)	potent neutralisation of a broad range of primary isolates of HIV	delayed seroconversion and decrease in the viral load of chimpanzees infected with primary isolates	Muster et al, 1994 D'Souza et al, 1997 Conley et al, 1996
IgGb12	gp120 (epitope overlapping the CD4 binding domain and the V2 loop)	potent neutralisation of a broad range of primary isolates of HIV	inhibition of primary isolates of HIV in hu-PBL/SCID mice	Burton et al., 1994 Gaudin et al., 1997 Kessler et al., 1997
2G12	gp120 (epitope overlapping the V3 loop and the V4 region)	potent neutralisation of a broad range of primary isolates of HIV		Trkola, 1996 D'Souza et al, 1997
694/98D	gp120 (V3 loop)	neutralisation of several laboratory strains of HIV, activation of complement		Gorny et al., 1993 Spears et al., 1993
F105	gp120 (CD4 binding domain)	neutralisation of several laboratory strains and primary isolates of HIV		Posner et al., 1993

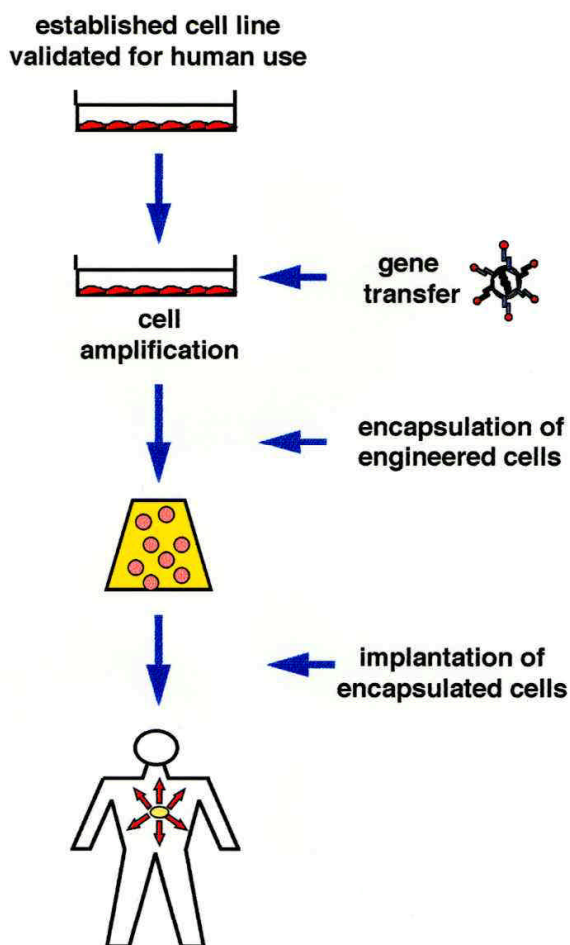
red for both production and correct folding of antibodies. So far, the production of antibodies by engineered cells has proved weak as compared to the production by cells of the B lineage. However, it is very likely that poor production results, not from the inability of the various cell types to make and secrete antibodies, but rather from poor expression of the retroviral vectors used for gene transfer. Improvement of the latter will thus constitute a major step towards efficient antibody-based gene therapy.

Figure 2. Systemic production of cloned antibodies through grafting of genetically modified myogenic cells. Primary myoblasts are isolated from mouse muscle biopsies and expanded *ex vivo*. Following retroviral gene transfer of the cloned monoclonal antibody, stably transduced cells producing the antibody are selected and amplified for implantation into recipient mice. Myogenic cells are grafted by simple injection into the *tibialis anterior* muscle of mice treated with cardiotoxin. The antibody produced is released into the bloodstream (For more details, see Noël et al., 1997; Pelegrin et al., 1998).



V. *In vivo* production of antibodies by encapsulated cells.

Systemic production of antibodies in mice implanted with cells encapsulated into various biocompatible materials has been achieved by several groups (Okada et al., 1997; Pelegrin et al., 1998; Savelkoul et al., 1994). In the context of gene therapy, capsules are interesting for at least two reasons. First, they constitute immunoprotective devices since the size of their pores can be adjusted in order to allow the diffusion of small molecules (such as nutrients and antibodies) through them but can prevent the passage of cells. In other words, encapsulated cells, which are efficiently retained within capsules, are protected from immune cells of the host which cannot enter the matrix. This property is important with regard to the versatility of the capsule approach since non-autologous, or even xenogenic, cells can potentially be implanted into individuals (Figure 3). Second, capsules offer an advantage with respect to safety since, in contrast to grafted cells, they can easily be removed by simple surgery if, for any reason, the treatment needs to be terminated.



Several types of polymers have been used to encapsulate antibody-producing cells for implantation into mice. These include cellulose sulphate (Dautzenberg et al., in press; Pelegrin et al., 1998), alginate (Savelkoul et al., 1994) and alginate-poly(L)lysine-alginate (Okada et al., 1997). The various matrices used differ in their physical and mechanical properties with cellulose sulphate (Dautzenberg et al., in press) offering advantages over the other two which either rapidly deteriorate or induce an inflammatory response, respectively. Interestingly, cellulose sulphate capsules (Figure 4) implanted subcutaneously form neoorgans which are extensively vascularized within days and are stable for at least 10 months (Figure 5) (Pelegrin et al., 1998). This is certainly beneficial for two reasons. Antibody uptake by the blood is favored and a better supply of nutrients is achieved, thus favoring cell survival in the capsules. Alternatively, other biocompatible immunoprotective devices, such as polyethersulfone fibers, might be used to replace capsules for implantation of antibody-producing cells *in vivo* (Déglon et al., 1996).

So far, only encapsulation of cells with short life-span within capsules, such as hybridoma cells, has been tested for transient antibody production *in vivo*. It will thus be crucial to test whether long-lived primary cells or cell lines can also be used for long-term production. This seems possible since primary skin fibroblasts have already been shown to survive longer than one year *in vivo* when encapsulated in alginate-poly-L-lysine alginate (Tai and Sun, 1993). Work is currently underway to address this issue.

VI. Overcoming some of the possible hurdles.

The possible development of an immune response against the ectopic antibody and/or the antibody-producing cells is a major threat for this therapeutic strategy. This response can thus potentially be cellular and/or humoral.

In case of grafting engineered autologous cells, a cytotoxic response against antibody-producing cells is very unlikely to occur; this is because secreted antibodies are not foreign molecules, provided they are of human origin or of the species in which the experiments are being conducted. However, it cannot yet be ruled out that ectopic

Figure 3. Systemic production of antibodies by implantation of encapsulated antibody-producing cells. Established cell lines validated for human use can be genetically modified to produce therapeutic antibodies upon gene transfer. Selected antibody-producing cells can be amplified and encapsulated in immunoprotective devices (see text and Dautzenberg et al., in press). Capsules are implanted subcutaneously by simple surgical treatment for systemic delivery of therapeutic antibodies.

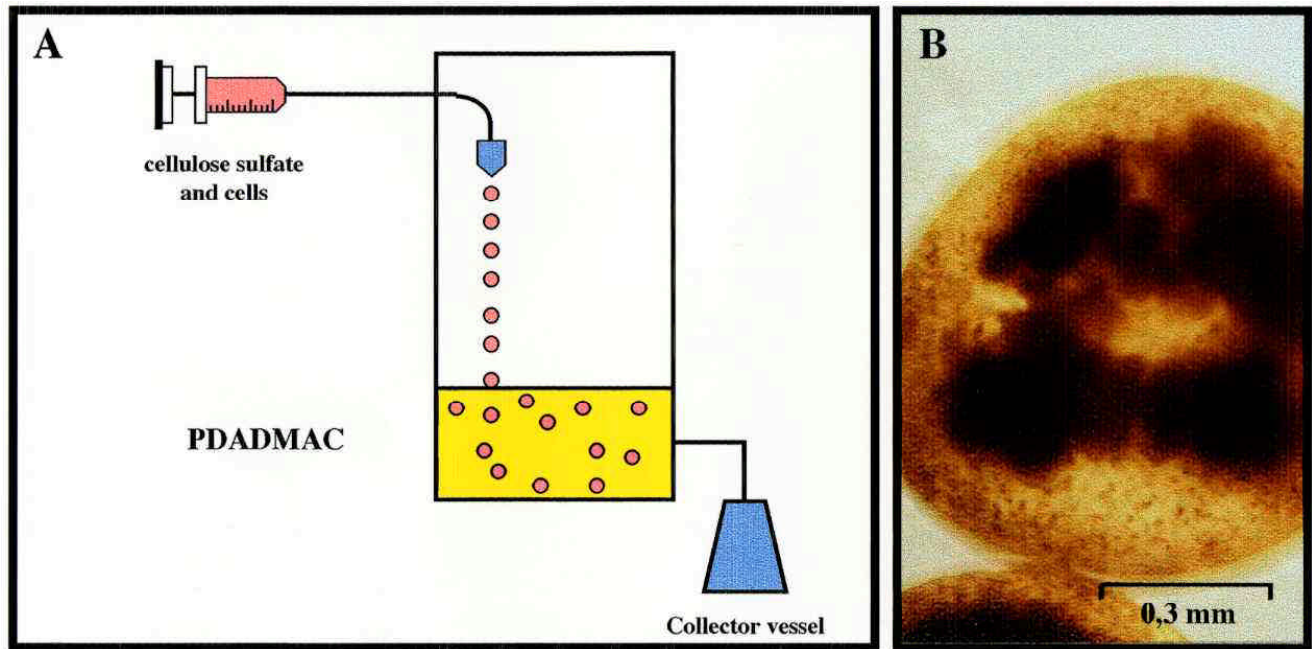
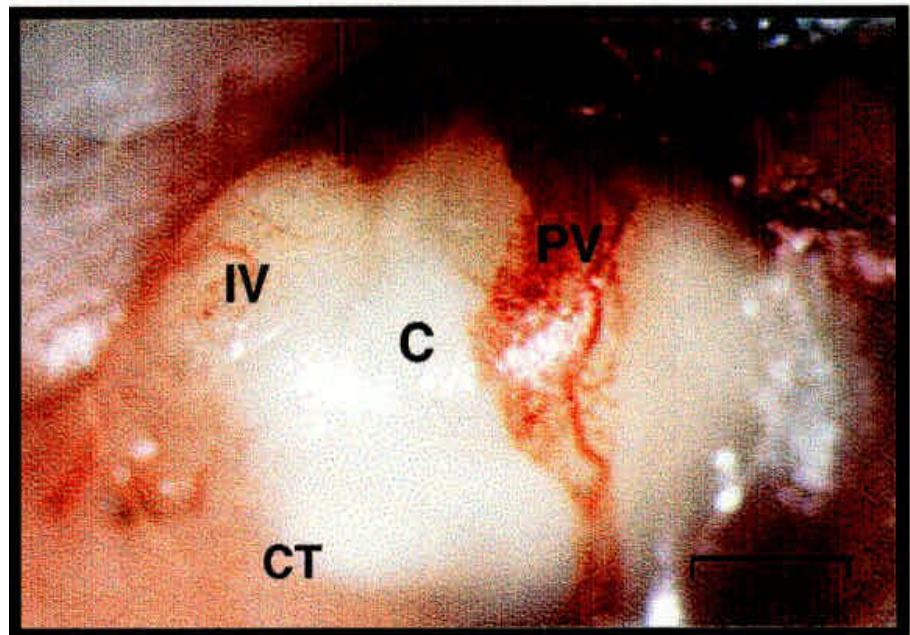


Figure 4. Cellulose sulphate capsules. **A.** *Production of cellulose sulphate capsules.* Cells are resuspended in a cellulose sulphate solution. Droplets of the suspension are generated and dropped into a solution of polymerization catalyst (PDADMAC). Capsules form within 90 second. After washing with the appropriate medium, they can be implanted immediately or kept in culture for several days to several weeks before use (Dautzenberg et al., in press). **B.** Cellulose sulphate capsules containing hybridoma cells. These capsules have an average diameter of 0.6 mm. The dark zones correspond to encapsulated cells

Figure 5. Neo-organ formation following implantation of encapsulated cells. Cellulose sulphate capsules containing antibody-producing cells are vascularised within a few days when implanted subcutaneously (Pelegrin et al., 1998). In this experiment, a group of 10 capsules (**C**) was implanted. Within 3 days they were wrapped in a pouch of loose connective tissue (**CT**) which rapidly underwent peripheral vascularization (**PV**). Later, blood vessels extended into the inner part (**IV**) of this pouch for irrigation of the neo-organ.



antibodies can be degraded producing antigenic peptides presentable by MHC class I molecules when expressed in non-B cells. If this occurs a cytotoxic T cell response against such cells could be triggered. This issue merits thorough analysis.

The situation is quite different in the case of capsule implantation : even if encapsulated cells are non-MHC-matched or xenogenic, they cannot be destroyed by host cytotoxic T cells because no physical contact is allowed between the two types of cells. However, xenogenic cells are sometimes killed by a mechanism involving complement-mediated lysis. An appropriate choice of xenogenic cells and/or adapted strategies for protecting cells from complement will, thus, be necessary. It is likely that cellular debris released from the capsules could trigger a cytotoxic T cell response directed against encapsulated cells. However, more than being a drawback, this response should present an advantage with respect to the safety: in case of accidental escape from capsules (after breakage, for example), antibody-producing cells released into the bloodstream would immediately be destroyed by circulating T cells.

A more serious threat is the possible generation of humoral anti-idiotypic responses against therapeutic antibodies. Such immune responses were observed in patients treated with repeated injections of high doses of purified antibodies. Sometimes, but not always, they could even neutralize the treatment (Isaacs, 1990). It must, however, be emphasized, that in no clinical trial performed so far, were the antibodies of human origin : at best, they were humanized murine antibodies. Moreover, it is not clear whether the observed anti-idiotypic responses were primary responses against the idiotypes of the injected antibodies or just parts of responses directed against whole non-self proteins.

In contrast with these observations, no detectable anti-idiotypic response was observed in mice producing a model anti-human thyroglobulin monoclonal antibody upon grafting engineered myogenic cells (unpublished data) or upon implantation of cellulose sulphate capsules containing hybridoma cells (Pelegri et al., 1998). Even though these first data are encouraging, such studies need to be extended to a number of other immunoglobulins to establish whether ectopic monoclonal antibodies produced *in vivo* are immunogenic or not. It is also possible that the concentration of antibody released systemically is crucial in triggering anti-idiotypic responses. In this case, determining the threshold levels of antibody required for the mounting of the immune response will be crucial for developing efficient long-term antibody-based gene therapies. Inducible expression systems, such as the tetracycline system of Bujard and co-workers, might reveal invaluable tools for adjusting the concentration of antibody delivered into the bloodstream of patients.

VII. Conclusions

Using model systems, we have demonstrated the feasibility of the *in vivo* production and systemic delivery of antibodies by engineered cells. Our work thus sets up the technical basis for a new gene/cell therapy approach aimed at the long-term treatment of patients suffering from a variety of severe diseases such as cancer, viral diseases and various autoimmune diseases.

The two major issues which must now be solved before application of this novel therapeutical strategy to humans are, (i) the optimization of the *in vivo* production of antibodies and, (ii), the validation of its therapeutical value in several animal models of human diseases.

For optimization in antibody production, several approaches have already been considered. The use of cell lines certified for human use and amenable to encapsulation certainly constitutes a promising approach for various reasons including efficiency, cost-effectiveness and safety. Nevertheless, using *in vivo* injectable vectors, such as adenoviruses and AAV, for long-term production of monoclonal antibodies *in vivo* is also a promising approach. Finally, we have recently been able to protect mice from developing a lethal retroviral disease using systemic delivery of antibodies by antibody-producing cells, thus providing the first demonstration of the therapeutical potential of the approach. Extension of this study to other animal diseases is currently under investigation and should pave the way to human applications.

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