

The effect of interferon- γ on genetic immunization

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The effect of co-inoculation of a plasmid vector expressing the rabies virus glycoprotein and an additional vector encoding mouse interferon (IFN)- γ on the development of an antigen specific B and T helper cell response was tested upon intramuscular inoculation of mice. The effect of IFN- γ was dependent on the promoter driving expression of the viral antigen. The immune responses to antigen-expressing vector carrying a viral promoter such as the SV40 early promoter or the major histocompatibility (MHC) class I promoter were reduced in presence of IFN- γ while the B and T helper cell response to a vector expressing the antigen under the control of the MHC class II promoter was not affected by this cytokine. © 1997 Elsevier Science Ltd

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Genetic immunization, a recently developed method of vaccination that utilizes plasmid vectors expressing the antigen of a pathogen, evolved originally from gene therapy. Inoculation of vectors into the muscle of mice was shown to cause transfection of cells close to the inoculation site. Transfected cells in turn express the vector encoded proteins which, provided they are foreign, initiate an immune response¹ consisting of antibodies, T helper cells and cytolytic T cells. Vectors encoding antigens from mouse pathogens induce protection to challenge thus demonstrating that plasmid vectors have potential as vaccines.

In addition, the use of plasmid vectors as antigen substitutes provides immunologists with a powerful tool to study some of the rules that govern primary immune responses. Vectors can be constructed with comparative ease. The inserted gene can be modified by site-directed mutagenesis, by truncation, deletion, or insertion of sequences. The expression level of the antigen can be varied by changing the regulatory element; the location of expression can be restricted to certain cell types by using tissue-specific promoters. The duration of expression can be altered by the use of inducible promoters.

We have employed genetic immunization to test the effect of cytokines on the primary immune response to a vector encoded antigen. The effect of cytokines on the immune system has been tested by a number of methods all of which have limitations. Cytokines have been analyzed *in vitro* using established T cell lines or clones. Already activated lymphocytes respond to stimuli differently from naive cells. This approach thus fails to permit conclusions on the effect of cytokines during a primary immune response. As an alternative, antibody-mediated depletion or systemic supplementation of a cytokine

concomitant with administration of an antigen has been used. Most cytokines have pleiotrophic effects and changing the overall cytokine concentration of an organism is likely to have side-effects that bias the reaction under investigation. Genetically engineered mice that overexpress or completely lack a given cytokine have yielded interesting data but have shown overall that the immune system is exceedingly redundant and likely to use alternate pathways if deprived of an important substance. Cytokines act locally at the site of antigen infestation. None of the methods described above is technically suited to provide cytokines to a restricted area; this can be achieved by genetic vaccines. We initially used this approach to test the consequence of increased levels of granulocyte macrophage-colony stimulation factor (GM-CSF) on genetic immunization. The model we have been using is based on a plasmid, pSG5rab.gp, expressing the full-length rabies virus glycoprotein (G protein) under the control of the Simian virus (SV) 40 promoter. Mice were inoculated with a mixture of the pSG5rab.gp vector and another vector expressing mouse GM-CSF. Mice that were given the GM-CSF vector developed an enhanced T helper and B cell response to the rabies virus G protein during the early phase after immunization². This presumably reflected the GM-CSF triggered activation of local dendritic cells that are known to play a pivotal role in presenting antigen to virgin T cells. Mice immunized only with the pSG5rab.gp vector show a slowly increasing antibody response; peak titers are generally reached 10–12 weeks after a single inoculation and then persist at constant levels for at least 11 months (the latest time we have tested thus far). The kinetic was clearly changed after co-inoculation of a GM-CSF-expressing vector. Although mice initially developed substantially higher antibody titers to the rabies virus G protein, these titers were transient and eventually, after several months, declined below those of control mice inoculated with the pSG5rab.gp vector only.

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Table 1

Vector name	Promoter	Expressed protein
pSG5rab.gp	SV40	Rabies virus G protein
pCI5rab.gp	MHC class I	Rabies virus G protein
pCII5rab.gp	MHC class II	Rabies virus G protein
pSV2mu γ II	SV40	Mouse IFN- γ

In the next set of experiments, we tested the effect of interferon (IFN)- γ on the immune response to genetic immunization. We chose IFN- γ for it is known to upregulate expression of major histocompatibility (MHC) class I and II determinants on a number of cells including muscle cells, the predominant reservoir for vector encoded antigen. In the initial experiments groups of C3H/He mice were injected with a mixture of pSG5rab.gp and the pSV2mu γ II vector (kindly provided by Genentech, Inc., San Francisco, CA). Both the T helper cell response and the antibody response to rabies virus were slightly but consistently reduced in presence of IFN- γ thus refuting the notion that IFN- γ induced upregulation of MHC antigens on muscle cells improved their capacity to present antigen. One potential explanation for the observed reduction in the immune response might have been a negative effect of IFN- γ on the viral promoter driving expression of the rabies virus G protein. Although *in vitro* IFN- γ even when used at high concentration was not found to influence the expression of the rabies virus G protein on cells stably transfected with the pSG5rab.gp vector (data not shown), the conditions affecting regulation of integrated genes might differ from those ruling expression of proteins encoded by episomally retained plasmids. To further address this question we modified the pSG5 vector by replacing the SV40 promoter with an MHC class I or II promoter which encode determinants that are both upregulated in presence of IFN- γ . The biological activity of the two constructs was initially confirmed with the β -galactosidase reporter gene by transient transfection of either fibroblasts (for the MHC class I promoter) or macrophage lines (for the MHC class II promoter). The vectors were subsequently reconstructed to express the rabies virus G protein (vectors used for the *in vivo* studies are listed in Table 1). Mice inoculated with the plasmid expressing the rabies virus G protein under the control of the MHC class I promoter (pCI5rab.gp) which has broad tissue specificity developed an antibody response that was comparable in magnitude to that seen in mice immunized with the pSG5rab.gp vector. The B and T helper cell responses to the rabies virus G protein by the pCI5rab.gp vector were reduced upon co-inoculation of pSV2mu γ II thus indicating that the negative effect of IFN- γ was unlikely to reflect down-regulation of viral promoter activity.

Expression of the rabies virus G protein under the control of the MHC class II promoter by the pCII5rab.gp vector resulted in a comparatively weak immune response (Figures 1 and 2). Expression of the G protein under the control of the MHC class II promoter should limit synthesis of the protein to cells that constitutively express MHC class II molecules such as dendritic cells, macrophages and B cells. As professional antigen-presenting cells, crucial for initiation of an immune response, especially the MHC class II restricted CD4⁺ T helper cell response, belong to this group, the

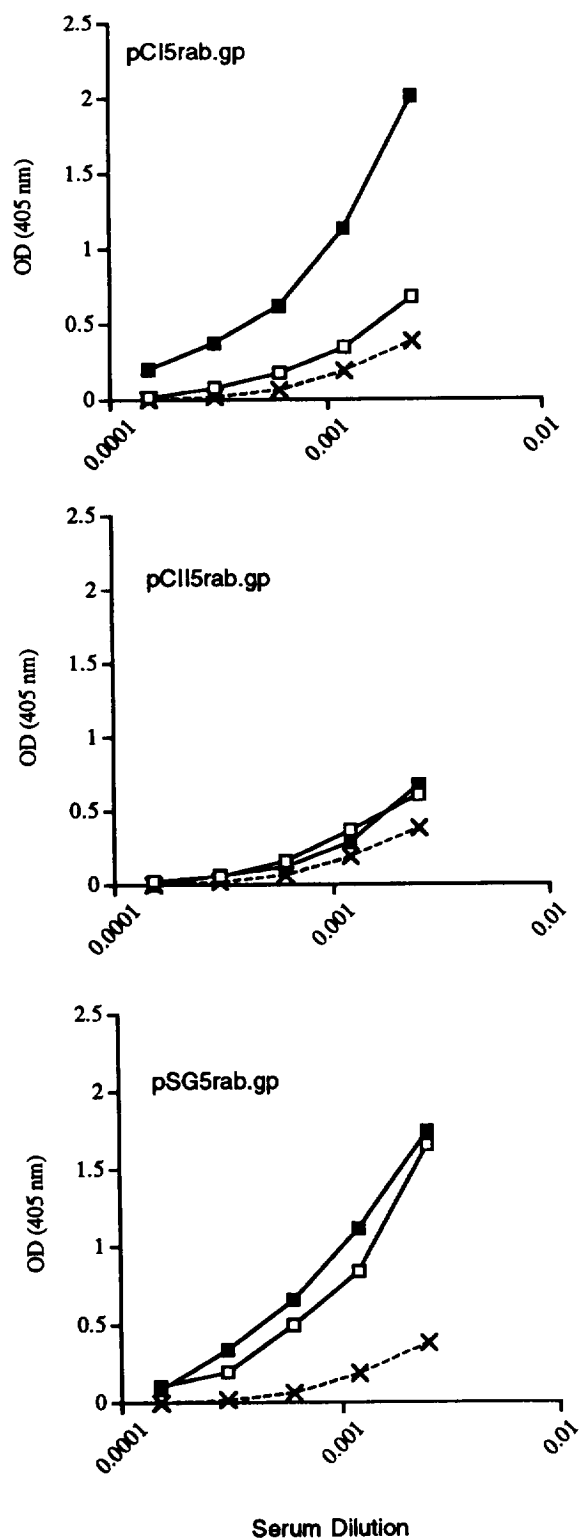


Figure 1 Effect of IFN- γ on the B cell response to rabies virus. Groups of mice were inoculated i.m. with 50 μ g of either pSG5rab.gp, pCI5rab.gp or pCII5rab.gp with or without 50 μ g of the pSV2mu γ II plasmid. Mice were bled 6 weeks later and serum antibody titers were analyzed by an ELISA as described⁹. \times , Normal mouse serum; mice immunized with: antigen expressing vector, i.e. pSG5rab.gp, pCI5rab.gp or pCII5rab.gp, alone (\blacksquare), antigen expressing vector+pSV2mu γ II (\square)

low stimulation of B and T helper cells upon inoculation of the pCII5rab.gp vector might reflect that this promoter is weak compared to the SV40 or the MHC class I promoter. Alternatively, the data might indicate that direct transfection of MHC class II⁺ cells such as dendritic cells suffices to initiate a weak immune

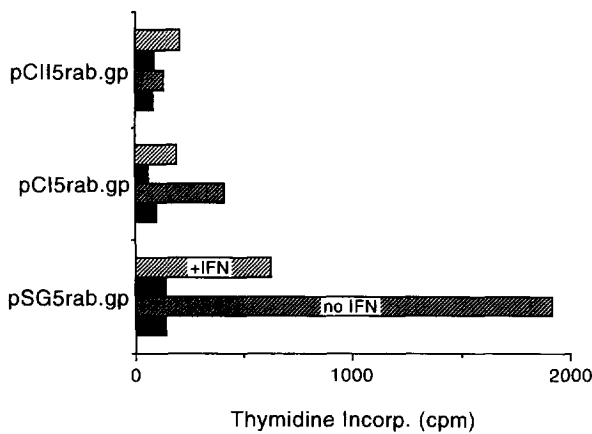


Figure 2 The effect of IFN- γ on the T helper cell response to rabies virus. Mice immunized as described in legend to *Figure 1* were euthanized 6 weeks after immunization and splenocytes were tested for cytokine release upon co-culture with medium or inactivated rabies virus as described previously⁴. Antigen-expressing vector only (▨, ■); antigen-expressing vector+pSV2mu γ II (▩, □); Lymphocytes co-cultured with medium (■, ▩); lymphocytes co-cultured with antigen (▨, ▩)

response but that additional antigen, provided by MHC class II⁻ cells such as muscle cells is required to augment the response. Co-inoculation of mice with the vector expressing the rabies virus G protein under the control of the MHC class II promoter together with the IFN- γ encoding plasmid did not result in inhibition of the albeit low immune response suggesting that down-regulation of the immune response by this cytokine might depend on the cell type serving as the primary source of antigen.

We have presented data here and in a previous manuscript showing that the method of genetic immunization can be used to elucidate the role of cytokines during a primary immune response. These studies are at the beginning and have to be carried out in more depth: dose response curves have to be conducted to reveal if cytokines have different activities depending on local concentrations; the influence of the genetic background of the host has to be taken into account, and the kinetic of cytokine activity should be analyzed by the use of inducible promoters.

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