Introduction:
gene vaccination, current concepts and future directions

Eyal Raz

Department of Medicine, University of California San Diego, 9500 Gilman Drive,
La Jolla, CA 92093-0663, USA

Gene vaccination: the phenomenon

Intramuscular (i.m.) inoculation with plasmid (p) DNA encoding a specific antigen (gene vaccination) has been shown to induce both antibodies and cytotoxic T lymphocyte (CTL) responses [12, 23, 37, 40, 41]. In different models of viral diseases, in which challenge of immunized animals with virulent virus is possible, these responses have been found to be protective [28, 37]. Antibodies have been raised in various species (e.g., chickens, mice, ferrets, cattle and non-human primates) by the injection of pDNA that encode various antigens such as: hemagglutinin [37], matrix protein and nucleoprotein from the influenza virus [9], glycoprotein (gp) 120 and pg160 from the HIV-1 ([40, 41] and Kim et al. in this issue), glV from bovine herpes virus [7], surface gp from rabies virus [43], hepatitis B virus (HBV) surface antigen ([8, 14] and Davis et al. in this issue), the malaria sporozoite protein ([16] and Hedstorm et al. in this issue), the heat-shock protein (hsp) 65 antigen from mycobacteria tuberculosis ([36] and Lowrie et al. in this issue), and hepatitis C virus (HCV) core antigen ([15] and Inchauspe in this issue). The immune response induced by injection of pDNA-encoded viral antigens persisted more than 12 months in Rhesus monkeys [9, 40, 41].

Gene vaccination has also been successfully utilized to elicit anti-tumor [4], anti-idiotype [35, 42], and alloimmune responses [13], to suppress an autoimmune disease such as experimental allergic encephalitis [39] as well as to inhibit IgE formation and allergic responses ([17, 29, 30] and Roman et al. in this issue).

However, muscle is not considered the best site for antigen presentation because it contains few, if any, dendritic cells (DC), macrophages and lymphocytes [28]. The skin and mucous membranes are the anatomical sites where most exogenous antigens are normally encountered [33]. The skin-associated lymphoid tissue contains specialized cells which enhance immune responses [34]. The keratinocytes produce interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and IL-12 [1], which can activate macrophages, lymphocytes and DC. The Langerhans’ cells (LC) of the skin carry the antigen from the skin to the draining lymph nodes. Antigen-loaded antigen-presenting cells (APC) such as LC or DC are potent activators of naive T lymphocytes. Furthermore, it has recently been shown that in gene-vaccinated animals, bone marrow-
derived cells (e.g., APC) and not somatic cells (e.g., myocytes) dictate the nature of the subsequent immune response to the gene product ([15] and Corr et al. in this issue). Therefore, the in vivo transfection of dermal and epidermal cells, and especially APC, would be expected to provide an efficient route for gene immunization that mimics the physiological response to viral infections of the skin, as has recently been demonstrated [4, 28]. Indeed, intradermal (i.d.) injection of pDNA encoding influenza nucleoprotein (NP) or Escherichia coli β-galactosidase (β-gal) in a human cytomegalovirus-based expression vector led to prolonged intracellular antigen expression by keratinocytes, fibroblasts and cells with the morphology of macrophages and DC in the dermis [5, 28]. The i.d. gene vaccination induced both antibodies and CTLs specific for the gene product which persisted for at least 17 months post-inoculation [28], and protected the gene vaccinated mice from lethal challenge by the influenza virus [28]. Thus, i.d. or i.m. gene vaccination are both effective routes for the induction of antigen-specific humoral and cellular immune responses.

Analysis of the T cell response to the gene product induced by the i.m. and the i.d. routes has shown that gene vaccination elicits an antigen-specific Th1 response to the encoded antigen [22, 29], whereas gene gun immunization generates Th0/Th2 immune response [10]. The induction of a Th1 response to protein antigens, such as allergens, has been used to down-regulate IgE and allergic responses (see Roman et al. in this issue).

The adjuvanticity of DNA: the role of the immunostimulatory DNA sequences

One of the most intriguing questions that is frequently asked is “Why does gene vaccination which generates only picograms or nanograms of gene product (antigen) induces a strong immune response that is usually a Th1 response, whereas immunization of animals with the same dose of antigen (picograms or a few nanograms) does not elicit any response at all”?

In animals models potent adjuvants magnify the immune response to low concentrations of the injected antigen. However, since gene vaccination is usually employed without any adjuvant, as “naked” pDNA (dissolved in normal saline), it was difficult to understand why it is such an effective vaccine. It was then discovered that certain DNA sequences in the pDNA backbone act as adjuvants and may control the nature of the subsequent immune response to the gene product [20, 31].

Several types of polynucleotides are mitogenic for lymphocytes. For example, polymers composed of guanosine and cytosine (poly G:C), or inosine and cytosine (poly I:C), are potent inducers of interferon-α (IFN-α) and activate the lytic potential of macrophages and natural killer (NK) cells [46]. By synthesizing oligodeoxynucleotides (ODN) from different regions of the mycobacterial genome, single-stranded 45mer ODN were identified which also activate macrophages and NK cells. This cell activation was attributed to DNA sequences containing a CpG motif within a palindromic hexamer that follow the formula: 5’-purine-purine-CG-pyrimidine-pyrimidine-3’ (e.g., 5’-GACGTC-3’, 5’-AGCGCT-3’ and 5’-AAGCGT-3’) in the ODN [45]. Bacterial DNA (but not vertebrate) was also shown to induce B cell proliferation and immunoglobulin secretion [24]. The mechanism of this B cell stimulation has been unclear. Structural differences between bacterial and vertebrate DNA such as the presence of adenosine or cytosine methylation in the vertebrate genome, and/or the absence of CpG suppression in bacteria, were claimed to be responsible for the observed phe-