Report of the Meeting on Genetic Vaccines (Naked DNA/RNA) WHO Geneva, May 17-18, 1994

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The idea of injecting naked DNA to get antigen production, and trigger the immune response, was reborn after the experiments conducted by Jon Wolff's Laboratory in the University of Wisconsin at Madison, and was ably commented by Jon Cohen (1993), and Thomas Braciale (1993). Several years ago, Benvenisty and Reshef (1986), emitted the astonishing report that viral and cellular gene promoters fused to the chloramphenicol acetyltransferase gene (CAT) and the hepatitis B surface antigen (HBSA), when injected into liver and spleen of newborn rats, would result in the expression of CAT and HBSA. No other reports appeared until the *Science* 1990 paper by Wolf et al. (1990). Here, DNA and RNA expression vectors containing genes for CAT, luciferase and β-galactosidade injected into mouse skeletal muscle *in vivo*, expressed proteins with no requirement for special delivery systems. The key issue in this new approach rests in the assumption that, when vaccination with purified proteins or peptide is carried out, these molecules are degraded by macrophage's endosomal cell system, and presented afterwards at the surface for lymphocyte recognition. Live virus vaccines are best, because the proteins are expressed inside the cells and folded and presented efficiently at the cell's surface. Thus, since in the DNA vaccines the proteins are properly expressed and correctly presented by the muscle cells (from within) they should resemble live vaccines. In order to review the current status of the field, and discuss the latest developments in this new approach to vaccination, the Global Programme for Vaccines (GPV), the Special Programme for Research and Training in Tropical Diseases (TDR), and the Global Programme on AIDS (GPA), jointly organized the Genetic Vaccines Meeting.

The Meeting was divided into six parts: General issues, Vaccine potential, DNA vaccines, RNA vaccines, Safety and regulatory issues, and Specific vaccines. The speakers were leaders of the respective fields. In the first section Professor Zinkernagel from the University of Zurich, reviewed the basis of vaccination, covering such subjects as antigen presentation, tolerance, memory and immunogenetic capacity in different systems. This talk was enlightening since it addressed the many questions raised by this new technology, its advantages and potential dangers. I will refer to the few critical issues I derived from this and following talks. Most of the experimentation has been carried out injecting muscle (so far the best target), shooting the skin and or spraying the nasal mucose. As the immunology dogma goes, miocytes or keratocytes are not considèred to be typical antigen-presenting cells. Therefore, either these cells are revealing unknown capacities, or a minor fraction of other cells, such as skin Largerham cells, are responsible for this presentation. In the latter case, the large amounts of DNA injected are justified by the scarcity of target cells. Also, since we do not yet know how to calibrate the dose, and how to focus on target cells, how can we control antigen presentation, and avoid the generation of tolerance at suboptimum doses? This is particularly hot issue, since a large part of the vaccination programs are focussed on babies, whose immunological systems may not be fully developed.

An intriguing, yet unexplained fact, is that DNA/RNA vaccines activate mostly cytotoxic cells (cytotoxic T lymphocytes of CTL), a property highly regarded in antiparasitic vaccines.

In spite of the many doubts, the field has passed the stage of interesting observation, to became an increasingly practical reality. The majority of the viral vaccines tried, have succeeded in maintaining a sustained antigen production, eliciting both arms of the immune response. For influenza this is perhaps the best vaccine ever presented. The overall strategy is to couple a DNA segment coding for proteins of the capside-core or basic function proteins to a strong viral promoter, which in turn confers a wide-spectrum protection; a escape-free immune response, and a high level of the antigen expression. The DNA doses are high, ranging from 1 to 100 µg per shot, and the delivery is now being assayed through nasal sprays and skin shots (with a Jet-Gun developed at Agrocetus, and licensed to BioRad). New and better expression vector are being designed, and the basic mechanisms of DNA uptake, protein expression and presentation are under intense study, and the field of muscle immunobiology is widening.

Other viral vaccines are on the list, but the most wanted is AIDS; four speakers presented

advances on HIV vaccines. The newer approach was to assemble multicistronic constructs covering basic functions of the virus which in turn confers wide protective spectrum in monkeys.

Two vaccines against parasitic protozoa were discussed, *Plasmodium falciparum* and *Leishmania*. In the first, although antigen expression was low, some protection was achieved. A puzzling fact was the expression of the flanking rather than the central part of the sporozoite protein. For *Leishmania*, the antigen was the surface protease gp 63; the authors got a low level of expression and protection in experimental animals. These two examples illustrate the need to think more deeply on non-viral vaccines: firstly, it is likely that the low expression levels, and the unexpected expression patterns reflect differences in codon usage preferences, which are completely different in *Plasmodium* and *Leishmania*, as compared to the vertebrate host. Secondly, for obvious reasons, it does not seem to be wise to express whole proteases as antigens.

Few examples of RNA vaccines were discussed, the attractive on using RNA instead of DNA is its instability, since it would only be transiently expressed without the risks of integration. These vaccines are in a vey preliminary stage.

Biosafety issues were discussed extensively: the two main concerns were the probability of getting insertions of the injected DNA, and the possibility of raising anti-DNA antibodies. Many speakers addressed the first issue citing the use of PCR to rule out any integration into the recipient genome. In my view, even lacking experimentation, it has been shown that by using retroviral DNA, the probability of knocking-out a gene, or activating a protooncogene is very low (Temin 1990). Also, comparing vaccines using whole killed organisms, a large amount of proteins and DNA is injected, so that a higher concentration is used, but it is no less true, that within *Leishmania* there are highly repeated sequences, such as kDNA with ten thousand copies per cell.

As to the second issue, DNA is a very poor antigen; even after such massive injections (100 μg for mice or rats) no anti-DNA antibodies has been detected in various strains of rats and mice. It is likely that FDA and other agencies, will deal with DNA-vaccines case by case, and we may soon see the first human trials.

A very important aspect, included in the agenda but not discussed at the meeting, was the participation of the Third World. Naked-DNA vaccines have two very important attributes as "perfect" vaccine for under-developed countries: thermal stability and inexpensive production. In a modest laboratory, using standard techniques, milligrams of DNA can be easily produced, and DNA is very stable. I will add a third quality, i.e. it is soft-core technology, using very simple molecular biology protocols and unsophisticated apparatus. There is a large collection of good antigens already cloned in Third World laboratories that can readily be used.

A limitation to the technique is patent protection. This is now being claimed on very wide basis, that is, the concept of DNA-injection for protection. If granted in this maner, it would seriously hamper further development.

Finally, a long discussion was made about how the new technique of vaccination should be called, having in mind the negative public perception of genetic engineering. Several names were voted on, of which nucleic acid vaccines was the winner.

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