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COMMENT

Physical trauma of vaccination acts as a wake-up call to dangers in the skin

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In this issue of *Immunology*, Liu and colleagues have demonstrated for the first time a crucial role for the physical trauma caused by injection of DNA vaccines, in the enhancement of innate immune responses.[1] DNA vaccination delivers a plasmid encoding an antigen, usually administered intradermally. It has the advantages of being relatively cheap, of expressing only the relevant antigen within the host's own cellular machinery, and of lacking any of the potential health risks associated with live vaccines. The antigen is expressed either in resident keratinocytes from where it is taken up by antigen-presenting cells[2,3] or in the local dendritic cells themselves.[4] DNA vaccination elicits both cellular and humoral immunity[5,6] a good memory response, and has been shown to be protective against a number of infections.[7–9] However, the precise mechanism by which an antigen delivered in this way can trigger both innate and cognate immunity at the site of vaccination, and the mechanisms determining the type of immunity that results are still unclear. DNA vaccination is often less successful in man than in murine models and therefore understanding the immunology of DNA vaccination, to improve its efficacy, is an important goal.

Immunologists have employed a number of strategies to enhance the immunogenicity of DNA vaccines, to deliver the vaccine in such a way that antigen-presenting cells are most effectively targeted and activated. One strategy, for example, has been the use of 'genetic adjuvants', the inclusion of genes encoding cytokines, growth factors, or costimulatory molecules that are expressed at the site of injection, to enhance the immune response to the antigen. For example, inclusion of the genes for granulocyte–macrophage colony-stimulating factor,[10] interleukin-18 (IL-18),[11] IL-2, IL-15, IL-12 and FLt-3 ligand (Flt-3L)[12] greatly increases the magnitude of the response to antigens of interest.

However, some of the adjuvanticity of DNA vaccines lie in the properties of the plasmid itself, as a result of the presence of CpG motifs.[13,14] The deliberate co-administration of CpG-rich plasmids during DNA vaccination can be employed to greatly enhance the immune responses.[15]

CpG motifs are short sequences of DNA that have been used as strong T helper type 1 (Th1) adjuvants for protein-based vaccines.[16] CpG motifs are one of many 'pathogen-associated molecular patterns' or PAMPs, molecular signatures found in foreign organisms at a much higher frequency than in host cells, e.g. lipopolysaccharide, double-stranded RNA, flagellin and bacterial lipoproteins. Methylated CpGs seem to have been lost from the genomes of vertebrates (so-called CpG suppression); as a result, sequences of unmethylated DNA are found at a higher frequency in microbial genomes.[17] PAMPs are recognized by specific germ-line encoded receptors, which serve as an early warning system, triggering rapid innate immune responses.

Toll-like receptors (TLRs) make a family of highly conserved pattern recognition receptors that are able to distinguish a wide array of PAMPs. Among them, TLR9 detects CpG motifs following transport of CpG-DNA into endosomes.[18] Following ligand recognition, TLRs can trigger profound changes in antigen-presenting cells and by expressing particular combinations of TLRs different antigen-presenting cells can detect the nature of the invading organism. TLR9 ligation enhances dendritic cell function by up-regulating antigen presentation, increasing co-receptor expression and stimulating production of Th1 polarizing cytokines such as IL-12.[19] Consequently, CpG motifs have proved to be an extremely powerful Th1-promoting adjuvant.[16]

In this issue of *Immunology*, Liu and colleagues address how DNA vaccination with CpG motifs can cause activation of innate immunity in the skin.[1] The ability of the skin to respond to CpG was something of a puzzle, as although both TLR2 and TLR4 are expressed by resident keratinocytes, TLR9 is not constitutively expressed in the skin. The authors injected the ears of BALB/c mice intradermally with plasmid, and detected TLR9 mRNA using a semi-quantitative polymerase chain reaction. Surprisingly, the act of injecting the skin, either with plasmid or with saline alone, caused similar levels of TLR9 up-regulation. As the volume of saline injection was increased from 0 to 5, 10, 15 and 20 μl, the expression of TLR9 mRNA in the skin also increased. TLR9 mRNA expression was transient, appearing after only 6 hr and returning to baseline levels 12 hr later.

The authors wished to identify which cells were expressing TLR9 in the skin in response to the physical trauma of injection. Although no clear identification of the cells was made, saline-injected skin did induce a strong inflammatory infiltrate and *in situ* hybridization revealed clusters of small cells at the inoculation site that appeared to be positive for TLR9 mRNA. The authors suggested that the TLR9 expression was the result of infiltration by blood leucocytes that express TLR9, rather than the *de novo* synthesis by resident skin cells.

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The next set of experiments addressed whether the newly up-regulated TLR9 expression could respond to CpG motifs in the plasmid. The increased expression of TLR9 did mediate responses in the skin; induction of the pro-inflammatory cytokines IL-1β, IL-6, tumour necrosis factor-α and IL-12 was significantly higher in mice that had received CpG-containing sequences.

This work gives us a number of important insights into DNA vaccination and immunity in the skin. It reveals that vaccine administration into the skin delivers a combination of signals to the innate as well as to the cognate immune system. Among those signals is the physical damage to the skin that occurs following intradermal injection. This is required to make the skin responsive to the PAMP, the CpG motif, which in turn acts to enhance cytokine production at the site of injection. This might explain why gene guns have proved such a good delivery system for DNA vaccines. For gene gun vaccination plasmid is coated onto gold particles that are then propelled into the dermal layer using high-pressure helium. In this technique the dose of plasmid required to generate immunity is far lower than is necessary for intradermal injection. The authors speculate that their observations could thus explain the increased efficacy of gene gun delivery. It would be an obvious next step to examine whether gene gun immunization really does result in a greater increase in TLR9 expression in the skin.

This work also sheds some light on the nature of immunity in the skin. It implies that the ability of the skin to respond to various stimuli that trigger the innate immune response can be altered under particular circumstances. The skin is an important first line of defence against infection and this plasticity of response makes sense in terms of function. Following physical damage, the threat of infection from the outside world is increased. By up-regulating the expression of pattern recognition receptors, the skin enters a state of heightened alertness to the threat of foreign invasion.

We can speculate upon which mechanisms of tissue damage result in recruitment of TLR9-expressing cells from the blood. Could the TLR9-expressing cells be identified and are these the same cells that present the antigen? What are the chemotactic agents that recruit these cells into the tissues? Are heat-shock proteins released from the damaged tissues as an initial signal that all is not well? As well as a possible role in triggering recruitment of leucocytes, it has been proposed recently that heat-shock protein 90 can act as a ligand transfer molecule which could play a crucial role in the signalling induced by CpG-DNA.[20] Identification of the key mediators in the processes described in this paper would offer new candidates for inclusion in future vaccines that exploit the adjuvant effects of tissue damage.

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