Innate immunity—beginning to fulfill its promise?

Much vaccine research is directed toward the generation of more effective vaccines. By combining DNA vaccines with the innate immune system, a flu vaccine was designed that required only a single immunization.

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Immunological research has always been a dual challenge for scientists and physicians: discovering how the complex immune system works, and determining how these discoveries may be applied to improve the health of mankind. We have made good progress with respect to defining the rules by which the immune system works but we have found the application of this knowledge for improved healthcare difficult to achieve. Perhaps this is because it requires both an understanding of the immunological rules and the good fortune (or foresight) that they happen to describe a therapeutically relevant part of the system. The study by Ross *et al.*[1] is especially notable, therefore, because it belongs to the latter category of research. It provides evidence that understanding the rules of innate immunity can enable the construction of DNA vaccines that are more effective at eliciting a protective antiviral acquired immune response.

To write that the use of plasmid eukaryotic expression vectors for immunization is fundamentally altering the field of vaccinology is not an exaggeration. The advantages of DNA vaccines relative to protein-based vaccines are that they are relatively inexpensive, can be prepared rapidly, are stable and, most importantly, can elicit MHC class I–restricted T cell responses[2]. However, as with protein immunization, the antigen produced by DNA vaccines is not immunogenic unless innate immune responses also are elicited. The first innate immune reaction found to be relevant to this form of immunization was the response to nucelotide sequences containing unmethylated cytosine phosphate–guanosine (CpG) dinucleotides with flanking regions of two 5′ purines and two 3′ pyrimidines. These so-called CpG motifs are found in prokaryotic DNA at much higher frequencies than in vertebrate DNA. By an unknown mechanism they induce production of immune-stimulating cytokines by dendritic cells, macrophages and natural killer (NK) cells[3]. The presence of these sequences in a DNA vaccine, either as part of the antigen-encoding plasmid or in a separate plasmid or oligonucleotides, greatly enhances both antibody and cytotoxic T cell responses[4]. Thus this innate immune system (which is still being molecularly characterized) interprets the presence of CpG dinucleotides as evidence of an infection and instructs the acquired immune system, through the secretion of cytokines, to respond to any foreign antigen that is present within the microenvironment.

In contrast to the somewhat fortuitous discovery of the role of CpG dinucleotides in promoting the immunogenicity of antigen encoded by DNA vaccines, Ross *et al.* set out to determine whether complement, a more fully characterized element of the innate immune system[5], also has this function. Whereas CpG dinucleotides alter the local microenvironment of an antigen to denote the presence of an infection, the complement system directly alters antigen itself, thereby identifying it as being of microbial origin. This is accomplished by conversion of the C3 component to the activated C3b fragment by a highly specific protease that is selectively assembled on the surface microorganisms following primary recognition by natural IgM, a mannose-binding lectin or a pathway that detects the absence of sialic acid. C3b then binds covalently to the target against which complement has been activated, thus permanently tagging it for recognition by the host. This occurs in the acquired immune system when bound C3b is proteolytically processed to smaller fragments, termed C3dg and C3d, which serve as ligands for the receptor CR2 (CD21), and which resides on B cells and follicular dendritic cells. On B cells, CR2 is complexed with the costimulator CD19 enabling C3d-bearing antigen to coligate CD19 to the antigen receptor and amplify sigaling by up to three orders of magnitude. CR2 on follicular dendritic cells captures antigen to promote the germinal center reaction and B cell memory. It has been shown that together these functions of CR2 cause a model antigen that is artifically tagged with three copies of C3d to be immunogenic at a concentration that was 0.001% that of the least immunogenic dose of unmodified antigen.

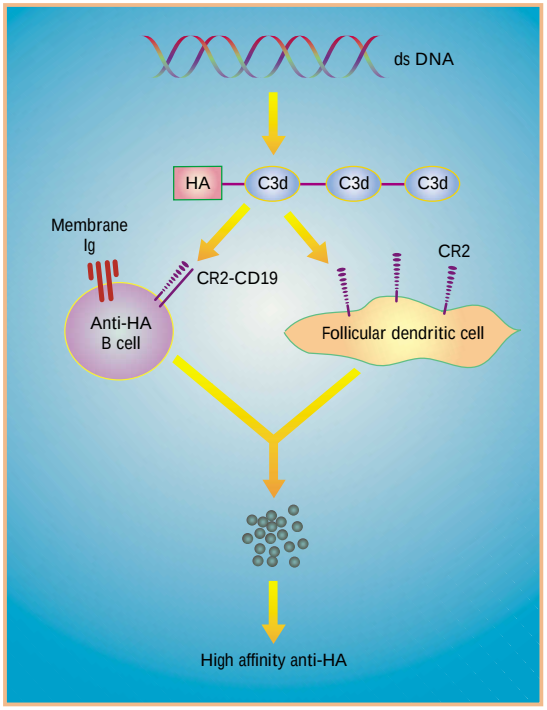

Figure 1. Model for vaccination with DNA-encoding influenza hemagglutinin (HA) attached to three copies of C3d. Cells take up the injected DNA and produce a fusion protein that interacts with HA-specific B cells and follicular dendritic cells (FDCs) via CR2. The cross-linking of the CR2-CD19 costimulatory complex to membrane immunoglobulin (Ig) on B cells enhances the germinal center reaction. HA on FDCs within germinal centers provides the source of antigen necessary for this reaction.As the germinal center is where memory B cells and long-lived plasma cells expressing high affinity antibody develop, these effects of tagging HA with C3d cause a vigorous and rapid humoral response that protects against infection.

Although this early work provided a sound rationale for the study by Ross *et al.*, no one had reported the ability of complement to augment the humoral immune response to an antigen that is relevant to a human disease, or to antigen administered as a DNA vaccine. It is striking, therefore,

that by attaching three copies of C3d to influenza, hemagglutinin improved the efficacy of the DNA vaccine by shortening the time required for immunized mice to acquire resistance to viral challenge and by eliminating the need for a secondary immunization. For combating rapidly emerging influenza variants, shortening the time in which immunity is acquired is important. The need for a single immunization only, if shown to be applicable to other antigens, would be especially desirable for vaccination programs in areas of the developing world where it might be difficult for individuals to return for booster immunizations. That C3d tagging may apply to at least some antigens relevant to infectious diseases in developing nations is suggested by the recent finding that mice immunized with a complex that consisted of *Plasmodium yoelii* merozoite surface protein fused to two copies of C3d were protected from infection, whereas those immunized with unmodified antigen were not[6].

As with all good studies, the findings of Ross *et al*. raise new questions. In addition to their discoveries about the effects of C3d, the authors found that anchoring influenza hemagglutinin to the plasma membrane also improved the immunogenicity of the antigen (measured either by antibody response or resistance to viral infection). The mechanism for this effect is as yet unclear; it would be interesting to see whether combining this modification with attachment of C3d would create an even better immunogen than either alteration alone does. The second observation that perhaps merits further investigation is that enhanced protection afforded by immunization with C3d-tagged hemagglutinin did not always correlate with antibody titers. The authors suggest that this finding could be explained by the more rapid affinity maturation of the antibody response elicited by C3d-bearing hemagglutinin. This would be consistent with the known role of CR2 in promoting the germinal center reaction, the site of somatic hypermutation of immunoglobulin genes. However, the antibody dependence of protection should be directly examined because another component of the antiviral immune response, such as cytotoxic T cells, may have been effectively recruited by C3d-bearing hemagglutinin. If this were the correct explanation the pay-off would be the discovery of a new function for C3d, as currently it is thought that C3d only promotes humoral immunity by enhancing B cell responses.

Recent interest in innate immune reactions has been rewarded with an improved understanding of how the acquired immune system is directed to antigens associated with infectious organisms. It is appropriate now that our understanding of the rules governing immunogenicity be tested for its relevance to improving vaccines, "a millennial challenge"[7]. The study by Ross *et al*. offers the encouraging thought that by focusing on innate immunity we may be on the right track.

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