Dendritic cell–based in vitro assays for vaccine immunogenicity

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Dendritic cells (DC) are pivotal in the induction of adaptive immune responses because they can activate naive T-cells. Moreover, they steer these adaptive immune responses by integrating various stimuli, such as from different pathogen associated molecular patterns and the cytokine milieu. Immature DC are very well capable of ingesting protein antigens, whereas mature DC are efficient presenters of peptides to naive T cells. Human DC can be readily cultured from peripheral blood mononuclear cells, which are isolated from human blood. There is a strong need to monitor in a high-throughput fashion the immunogenicity of candidate vaccines during the process of vaccine development. Furthermore, regulators require efficacy and safety testing for batch release. For some vaccines, these tests require animal testing, causing pain and discomfort, which cannot be contested because it would interfere with the test results. With the aims of promoting vaccine development and reducing the number of animals for batch release testing, we propose to use more broadly human DC for vaccine immunogenicity testing. In this commentary, this proposition is illustrated by several examples in which the maturation of human DC was successfully used to test for vaccine and adjuvant immunogenicity.

Introduction

Dendritic cells (DC) are sentinel cells that are able to initiate cellular immune responses.[1] They appear as immature DC which are very well capable of ingesting protein antigens and as mature DC which

are especially capable of presenting peptides to naive T-cells. Since, in contrast to T- and B-cells, DC are not antigen specific, DC-based assays do not measure antigen-specific responses but rather the response to pathogen associated molecular patterns (PAMP) associated with the vaccine (components). PAMP are detected by pattern recognition receptors (PRR) that are highly expressed by DC. PRR can be located on the cell surface, in endocytic compartments, or in the cytoplasm. Importantly, the nature of the ensuing immune response is greatly influenced by the PRR (or combination of different PRR) that is stimulated by the vaccine as well as the cytokine milieu. In this way, they form an important link between the innate and adaptive immune response. Several types of DC exist, the most common division being between myeloid DC and plasmacytoid DC. Myeloid DC (mDC) are specialized antigen-processing and -presenting cells, with high phagocytic activity as immature cells and high cytokine producing capacity as mature cells. They regulate T-cell responses both in the steady-state and during infection. Plasmacytoid DC (pDC) are specialized to respond to viral infection with production of type I interferons. They can, however, also act as antigen presenting cells and control T-cell responses.

In the process of vaccine development, monitoring their immunogenicity is crucial. For instance in case of allergy vaccines, many vaccines (allergens) are being developed of which only very few are sufficiently immunogenic. Testing all these "lead" vaccines would require many experimental animals. Furthermore, for batch release, regulators require that the

efficacy and safety of each vaccine batch be tested. For some vaccines, these tests require large numbers of animals, of whom a considerable number experience pain and discomfort, which cannot be contested because it would interfere with the test results.

DC play an important role in the induction of protective immunity by vaccines. It may therefore seem obvious to try to employ DC to evaluate vaccine immunogenicity and to do so by measuring DC maturation. Human DC can be readily obtained from human blood, circumventing both cross-species extrapolation and the use of laboratory animals. Here we review several examples that show the successful use for vaccine immunogenicity testing of in vitro assays based on human DC, being DC maturation but also more advanced approaches such as endolysosomal degradation and DC/T-cell co-cultures. The aim of the present commentary is to advocate the use of human DC based assays to evaluate vaccine immunogenicity for vaccine development and as alternative for animal testing for batch release.

Examples of In Vitro Assays Employing Dendritic Cells

Distinguishing the various components of the Hib vaccine. Comparison of various cell lines with monocyte-derived DC (moDC) by gene profiling revealed that the MUTZ-3 cell line most closely resembles moDC.[2] Using this cell line we were able to show differences in surface marker expression and cytokine production after in vitro treatment with the *Hemophilus influenzae* type b (Hib) antigen polyribosyl ribitol phosphate (PRP), the outer membrane protein of *Neisseria meningitides* (OMP) and a conjugate between PRP and OMP.[3] This conjugate is the actual vaccine; comparing the conjugate to the final product (with aluminum as adjuvant) did not show differences in surface marker expression or cytokine production. Although the conjugation process is routinely monitored biochemically, implicating that there exists no actual need to monitor this process using an in vitro assay, the method does show a proof-of-principle that different vaccine components can be distinguished using this in vitro assay.

The different responses between the vaccine components could also be shown rather similarly using moDC. Advantages of the MUTZ-3 cell line are that it is readily available, safe and shows minimal variability. Advantages of the moDC are its wide dynamic range compared with the MUTZ-3 cell line and the fact that it has a functional Toll-like receptor (TLR)4, whereas the MUTZ-3 cell line shows a poor signal transduction from TLR4.[4,5]

In conclusion, based on their immunogenicity the sugar antigen PRP, the bacterial antigen OMP and the conjugate PRP-OMP, could be distinguished using MUTZ-3 cells and moDC.

Efficacy of the YF-17D live attenuated yellow fever vaccine. The live attenuated yellow fever vaccine YF-17D is a highly effective vaccine. Its effects on moDC maturation in vitro were evaluated.[6] YF-17D efficiently induced CD80 and CD86 expression, and induced production of IL-6, TNF-α, MCP-1 (CCL2), IP-10 (CXCL10) and IL-12p40. IL-12p70 was also induced, but this required CD154 addition. Moreover, YF-17D induced IFN-α production by human pDC.

This study confirmed the high efficacy of YF-17D. To investigate the underlying mechanism, the effects of YF-17D on DC from Tlr knockout mice were studied. Mice deficient in Tlr2, Tlr7 or Tlr9 showed a clearly reduced IL-12p40 production, suggesting that these three Tlr act in a synergistic manner.

In conclusion, its effects on surface marker expression and cytokine production confirmed the high efficacy of YF-17D.

Assessing adjuvant immunogenicity. The CoVaccine HT™ adjuvant increased CD83 and CD86 expression on moDC, but not the expression of CD11c, CD80, or MHC II.[7] In addition, incubation with CoVaccine HT™ adjuvant increased IL-6 production and decreased IL-10 production, while production of IL-1β, IL-12p70 and TNF-α was unaffected. Production of IL-6 was almost completely abrogated by anti-TLR4 antibodies, but not by anti-TLR2 antibodies. Along this line, the decrease in IL-10 was reversed by anti-TLR4 but not anti-TLR2 treatment.

In this study in vitro DC testing proved to be valuable to screen for immunogenicity of the adjuvant. Such a screening would allow comparing the effects of different adjuvants and potentially safety issues related to adjuvants.

Pre-treatment with IFN-β enhances the immunogenicity of BCG. *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) provides variable protective immunity, urging the need for new tuberculosis vaccines. BCG infected DC showed increased expression of CD38, CD83, CD86 and HLA-DR, although this increase was lower compared with *M. tuberculosis* infected DC.[8] Furthermore, IL-12p70 production and IL-12p35 and IFN-β expression was lower by BCG infected compared with *M. tuberculosis* infected DC. Expression of IL-12p35 is regulated by IFN regulatory factor-3 (IRF-3) and it was indeed shown that IRF-3 phosphorylation was induced by *M. tuberculosis* infection but not BCG infection.[9] Pre-treatment of DC in vitro with IFN-β resulted in a much stronger expression of CD38, CD83 and CD86 and production of IL-12p70. Finally, IFN-β pre-treatment of DC resulted in increased production of IFN-γ and TNF-α by naive allogeneic cord blood lymphocytes.

This study shows that the in vitro DC assay can also be used to test for improving vaccine immunogenicity by adding cytokines, providing clues to improve vaccines for use in vivo in humans.

Endolysosomal degradation as a measure of vaccine immunogenicity. The rationale underlying the method of Egger et al.[10] is the link between the immunogenicity of a protein and its lesser susceptibility to endolysosomal proteolysis.[11,12] Using groups of structurally related proteins with different abilities to induce a T cell response in vivo, the authors were able to show a link between T cell priming in vivo and susceptibility to endolysosomal proteases simulated in vitro. The results obtained were similar using moDC and murine bone marrow-derived DC. Therefore, the authors also used the murine DC cell line JAWS II.[13] This cell line showed comparable results.

This is a very promising approach for immunogenicity testing. It would seem very useful to do a side-by-side comparison

between this approach, and the measurement of DC maturation.

Measuring vaccine immunogenicity using a co-culture of purified DC and naive CD4+ T cells. In bulk peripheral blood mononuclear cells (PBMC), DC may be present in too low numbers or in an activation state unsuitable for inducing a primary T cell response.[14] The authors therefore used a combination of purified DC and CD4+ T cells.

The protocol consisted of pulsing DC with YF-VAX® (a commercial preparation of YF-17D), co-culturing the DC with CD4+ cells in vitro, for 14 d and re-stimulation with YF-VAX for 7 h, after which the percentage of CD4+CD154+IFNγ+ cells was established. This protocol resulted in an increase in the percentage of positive cells from 0.4% to 3.2%. Remarkably, YF-VAX® vaccination in vivo resulted 6 to 12 weeks after vaccination in only a 1.2–2.6 fold increase in the percentage of positive cells. Moreover, the in vitro protocol resulted in CD4+CD154+ cells that produced other cytokines beside IFN-γ, such as TNF-α, IL-5, IL-17 and IL-21. This protocol proved to have a much higher sensitivity for priming naive T cells than the traditional in vitro PBMC based assay, even after addition of DC to the PBMC.

Although this method was shown to be successful for a highly effective vaccine and its performance in lesser effective vaccines remains to be established, it seems a very promising approach to evaluate vaccine immunogenicity. An important advantage over other studies may be that it is not based on DC maturation, but on a functional consequence thereof, being CD4+ cell expansion. Again, it would seem very useful to do a side-by-side comparison between this approach, and the measurement of DC maturation.

Conclusions

From the examples discussed in this Commentary it may be concluded that in vitro assays using human moDC are well capable of measuring vaccine immunogenicity. Moreover, evaluating the effects on DC in vitro with respect to the known effects in vivo will contribute to our understanding of vaccine-induced immune responses. Next, these assays may contribute significantly to a reduction in animal testing in the vaccine development process as well as for batch release. In vitro assays may contribute to a reduction in the time and cost of immunogenicity testing, which is especially relevant in the development process.

For a proper appreciation of the value of DC-based assays, a range of different bacterial and viral vaccines, live attenuated and inactivated vaccines and adjuvants should be tested. These tests should include evaluation of the ability to detect differences in immunogenicity between vaccine batches, and the potential for high-throughput capabilities. More advanced approaches such as measuring endolysosomal degradation and employing DC/T-cell co-cultures should be compared side-by-side to DC maturation.

In summary, we propose that using human DC for in vitro immunogenicity testing should be evaluated more broadly with the aims of increased understanding of vaccine-induced immune responses, reducing the use of experimental animals and increased throughput.

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