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Antibody–dependent enhancement and vaccine development

'Vaccines are a routine medical intervention performed on healthy individuals, so ADE needs to be considered seriously during vaccine development to ensure that vaccines protect individuals and do not exacerbate disease following subsequent infections.'

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The development of protective antibodies has long been the basis for vaccine development against a large range of infective agents. However, the discovery of the phenomena of antibody-dependent enhancement (ADE) in the 1960s gave rise to the concern that the development of antibodies could, at times, exacerbate the reaction to a natural infection by the microbe. Clinical evidence of exacerbation of disease by previous vaccination, infection or the presence of maternal antibodies is available for some microbes and hypotheses have been made about other agents. The phenomena of ADE must be considered during the development of new vaccines for a range of infective agents to ensure that the vaccines are protective and do not harm the recipients, particularly in light of new insights into the alteration of intracellular signaling pathways post-Fc-γR engagement by a microbe–antibody complex.

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It is a long-held view in immunology that antibodies are of the utmost benefit in defending the host from microbial infection. Antibody responses to a pathogen are often measured as an indicator of a properly functioning immune system. The antibody response indicates that the infectious agent is recognized by the adaptive immune response, will be cleared from the host and the immune memory established. Since Jenner vaccinated James Phipps with cowpox in 1796 to protect against smallpox, vaccine strategies have sought to induce antibody responses and produce B-cell memory to protect individuals from future infections by the same microbe, modify the severity of the disease and reduce the transmission of microbes between hosts.

However, there is another perspective on this traditional view that has been around for over 30 years and has gained renewed energy over the past 5 years with a number of fresh insights. ADE has historically been associated with virus infection. Hawkes first described ADE with the arboviruses, Murray Valley encephalitis, West Nile, Japanese encephalitis and Getah viruses in 1964 [1]. He found that viral titers in cell culture were greater if the initial infection was in the presence of virus-specific antibody (usually immunoglobulin [Ig]G) at subneutralizing concentrations. Given that subneutralizing concentrations are a normal phase of antibody responses, there was understandable concern that a suboptimal antibody response may exacerbate infection and this disease, rather than protect the host.

Other viruses have since been found to also exhibit ADE. For example, the more severe responses to Dengue virus infection, Dengue hemorrhagic fever and Dengue shock syndrome were found to be 15–80-times more likely in secondary infections than in primary infections [2]. Furthermore, a prospective study in Thai children found that those with

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pre-existing antibodies that were capable of enhancing Dengue virus type 2 growth in human monocytes *in vitro* and were at a greater risk of more severe disease following a second infection with Dengue virus [3].

Heterotypic anti-Dengue-virus antibodies enhance the uptake of virions into monocytic cell lines and primary human monocytes *in vitro* through an interaction with cell surface Ig receptors [4]. Yang and colleagues found that interferon (IFN)-γ was the major cytokine expressed in Dengue 2 infections in the absence of heterotypic antibodies, but that IFN-γ production was suppressed in the presence of heterotypic antibodies [5]. Chen and colleagues found evidence of decreased IFN-γ levels in more severely affected patients, that is, those with Dengue hemorrhagic fever compared with those with Dengue fever [6]. Furthermore, the authors found that subneutralizing antibodies were able to enhance virus replication, interleukin (IL)-4 levels appeared to be increased in the ADE reactions and there was an association between ADE and an increase in prostaglandin (PG)E_2 [7]. Later work found that Dengue hemorrhagic fever patients had significantly higher IL-10 levels and a lower Th1 response [6]. Similarly, ADE has also been associated with neurovirulence of the arboviruses, yellow fever and Japanese encephalitis viruses in mice [8] and enhance the replication of tick-borne encephalitis virus *in vitro* [9]. *In vitro* studies [10,11] have linked ADE to a variety of HIV strains in different cell types, raising the possibility that antibodies produced in trial subjects in response to HIV vaccines could enhance future natural HIV infections. This concern has led to expert meetings to discuss the challenges posed by suboptimal vaccination responses to HIV vaccines [12].

Early trials with formalin-inactivated respiratory syncytial virus (FI-RSV) found that the vaccines not only did not protect children against RSV but also appeared to increase the attack rate and clinical severity of later natural infection with RSV [13–15], possibly contributing to some deaths [16]. It has been suggested that exacerbation of disease after RSV vaccination and, possibly also in the presence of maternal antibodies, was also due to ADE mechanisms during later natural infections with RSV [13].

Studies with BALB/c mice have been undertaken in order to elucidate the mechanisms of this response to vaccination. A similar enhancement is also seen when mice are sensitized with the RSV-G glycoprotein [16] or vaccinia virus expressing the secreted form of the RSV G glycoprotein [17]. The severe disease that followed challenge with RSV was associated with elevated levels of IL-4, -5, -13 and eotaxin [18]. Interestingly, the secondary response to RSV infection following vaccination with FI-RSV mainly utilizes IL-4-mediated mechanisms [17].

The recent insights into ADE have revealed the crucial role of intracellular signaling as an underpinning molecular mechanism that explains the high growth of progeny virus in ADE-infected cells postinfection. The signaling impact of ADE is remarkable in that it has been found to be highly specific for key defense pathways, allowing an early and temporary suppression of IFN, tumor necrosis factor (TNF) and inducible nitric oxide synthase (NOS2) expression, leading to a cellular environment less threatening to virus survival. This is a seminal development in the understanding of ADE. The traditional view is that enhanced virus growth post-ADE infection is a function of the enhanced uptake of the virus through the Fc-receptors (or in some cases, complement receptors); the observations on post-ADE infection intracellular signaling show another dimension, namely the specific suppression of antiviral protein expression. Work on Ross River virus (RRV) infection in macrophages linked the suppression of IFN, TNF and NOS2 to the disruption of signal transducer and activation of transcription (STAT) complexes, immunoregulatory factor (IRF)-1 and necrosis factor (NF)-κB [19,20].

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The modulation of cell signaling processes post-infection has also been observed for the intracellular parasite *Leishmania*. The primary hosts – vertebrates – are infected by sandflies. The parasite causes disease that can manifest in the skin, nasal–oral membranes or the spleen, liver or bone-marrow [21]. *Leishmania* cells have two morphologies depending on whether they exist in the vertebrate host or the sandfly vector. The vertebrate form has no flagellum (amastigote) and the sandfly form has an anterior flagellum (promastigote). The promastigote is introduced into the vertebrate during sandfly feeding [22] and then enters phagocytic cells, predominantly macrophages, through the fibronectin receptor, the mannose-fucose receptor and the complement receptors (CR)1 and 3 [23] and transforms to the amastigote. These then replicate inside the phagolysosome of the cell, are able to infect neighboring macrophages [24] and are responsible for sustaining infection [25].

Kima and colleagues demonstrated that maintenance of *Leishmania* infections was impaired in the absence of antibody, thus indicting a role for ADE in the pathogenesis of leishmaniases [23]. Amastigotes with host-derived IgG on their surface and these IgG-opsonized amastigotes have increased virulence. Miles and colleagues demonstrated, in humans and mice, that infection with *Leishmania major* in the presence of IgG-immune complexes results in an inability to resolve infection in the host [24]. Kane and Mosser proposed that IgG forms an immune complex with the amastigote, which then ligates to Fcγ receptors to induce IL-10 production in macrophages and, in turn, inhibits macrophage activation and increases parasite growth [22]. This is supported by the findings of Kima and colleagues that the Fcγ receptor is required for amastigote parasite entry [23]. Increased IL-10 has been associated with visceral and cutaneous leishmanias and IL-10 has been flagged as having an important role in the regulation of the immune response to this intracellular

parasite [22] and links ADE with the Th1/Th2 immune response. Uzonna and colleagues showed that pathophysiological states are associated with characteristic Th1 or Th2 immune responses and that mice infected with subclinical infections that maintained a Th1 immune response were resistant to a higher infectious challenge [26]. A Th2 immune response and the production of IL-4 and -10, increase disease persistence by antagonizing the capacity of macrophages to produce NOS2 and consequently generate NO [25]. Gantt and colleagues propose that NO and superoxide contribute to the intracellular eradication of *Leishmania chagasi* in both human and mouse models [27]. In light of these studies, an antibody-based vaccine may not be effective in controlling leishmanias and may, in fact, contribute to disease through the development of immune complexes. Furthermore, as suggested by Uzonna and colleagues, clearance of the parasite may lead to loss of immunological memory and therefore loss of resistance [26]. Vaccination leading to clearance of the parasite may require continual and frequent exposure to leishmanias antigens to maintain the Th1 response and immunity.

A critical feature of this observation was the parasite-associated enhancement of IL-10 expression, a finding that supported earlier observations with the RRV-macrophage model of ADE [20]. The RRV study proposed that the role of pathogen-mediated stimulation of IL-10 was to globally downregulate the expression of early inflammatory and antiviral Th1 cytokines, such as TNF [20]. Increased levels of IL-10 have also been observed for Ebola, Junin, Lassa virus and Yellow fever virus infections [28].

Post-ADE alterations to cell signaling are not simply a matter of neutralizing key cellular defense proteins, but also for potentiating the expression of other host genes that assist the survival of the microbe. So far, the pathogen-stimulated alteration of cellular signaling pathways are connected with intracellular microbes, which makes biological sense given the microbe lifecycle.

An ADE mechanism for bacteria has also been observed but the mechanism involved enhanced bacterial adherence to mucosal surfaces. *Streptococcus pneumoniae* is a Gram-positive coccus. Although a commensal organism of the nasopharynx, it is also a major opportunistic human pathogen [29]. A major function of IgA is to stop bacterial adhesion and, therefore, colonization of host mucosal surfaces [30]. A recent paper by Weiser and colleagues suggests that the specific antibacterial antibody, IgA_1, the major form of IgA in the upper-respiratory tract, was cleaved by pneumoccocal IgA_1 protease, resulting in a survival advantage for the bacteria [31]. The protease produced Fab (antigen-binding) antibody fragments, the variable regions of which have an ionic charge, increasing adherence to host epithelial cells by the IgA(Fab) complex. This antibody-mediated mechanism may allow pneumococcus to persist on mucosal surfaces in the respiratory tract for extended periods.

In terms of vaccination strategies, the role of ADE and the subsequent downstream impact on signaling inside the infected cells has a much broader consequence beyond the early, enhanced growth of the microbe. What impact the early alteration of innate immune protein expression has on future adaptive responses in terms of microbe/parasite clearance and the formation of immunological memory, is yet to be fully understood.

Early vaccination strategies for some microbes have been successful and have grown to produce highly significant results, for example, Jenner's original work with cowpox eventually led to the eradication of smallpox from the planet. However, many vaccine trials have been unsuccessful in producing the desired immunity and some have actually harmed participants. Although many vaccine failures are owing to a lack of antibody production or nonprotective antibodies being produced, ADE raises concerns that subneutralizing antibody production may enhance subsequent natural infections and increase disease severity. Vaccines are a routine medical intervention performed on healthy individuals, so ADE needs to be considered seriously during vaccine development to ensure that vaccines protect individuals and do not exacerbate disease following subsequent infections.

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