

Exploiting the Redundancy in the Immune System: Vaccines Can Mediate Protection by Eliciting 'Unnatural' Immunity

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The fields of vaccinology and immunology have had a long-standing and complicated relationship. The view that vaccinology is a branch of immunology is more perception than reality. Vaccinology is older, practical, and often idiosyncratic, with its prime focus on the development of functional vaccines that protect a host against a particular microbe. Immunology is younger, conceptual, and strives to explain how a remarkably complex system recognizes and protects against internal and external threats without committing suicide. Vaccinology has been largely microbe-centered, clinically successful, and mostly empirical, whereas immunology has been largely host-centered, having struggled to translate its theoretical success and potential into clinical applicability. In this commentary, we examine the divide between vaccinology and immunology and put forth the view that vaccines often protect against infectious diseases by eliciting 'unnatural' immunity: immune responses that differ from and may even counteract those that arise during the development of natural immunity to a microbe.

The success of vaccinology, often achieved through empiricism, poses a challenge to the theses that vaccine design should be centered around the study of the immune response and that an effective vaccine should recapitulate the normal immune response to the relevant microbe. The eradication of smallpox by vaccination with vaccinia virus is probably the greatest achievement of vaccinology. However, this success also illustrates the tension between vaccinology and immunology, because smallpox (caused by variola virus) was prevented by inoculating humans with a different virus, the agent of cowpox (vaccinia virus). The prevention of variola by deliberate infection with a different virus (vaccinia) would appear to run counter to the tenet that specificity is essential for protective immunity, which has been central to immunological thought for over a century. Although vaccinia-induced protection against variola can be explained by antigenic mimicry, or

similarities between the antigenic determinants of the variola and vaccinia viruses, antigenic cross-reactivity is often viewed by immunologists as an exception to the central importance of specificity in immunological responses. In fact, the immunological cross-reactivity which allows the immune response to vaccinia virus to protect against variola virus is not a general phenomenon. One herpes virus may not confer protection against another herpes virus and immunity to one serotype of strains of *Streptococcus pneumoniae* does not confer immunity to another. Cross-reactivity has at times been viewed as a deviation from the immunological ideal, particularly when it is held responsible for autoimmunity and Pauls Erhlich's 'horror autotoxicus', phenomena that exemplify how the immune response can go wrong (1).

Toxoid vaccines represent another remarkable success for vaccinology. Diphtheria and tetanus are caused by *Corynebacterium diphtheriae* and *Clostridium tetani*, two bacteria that cause disease by elaborating toxins that damage host tissues. Toxoids are heat- or chemically-inactivated toxins that retain immunogenicity, but lack the capacity of the native toxin to induce host damage. While the use of toxoid vaccines has practically eliminated diphtheria and tetanus in vaccinated populations, their success in preventing disease stands in stark contrast to the fact that recovery from diphtheria or tetanus does not reliably confer immunity to recurrence of disease. The absence of lasting immunity after recovery from disease may reflect the rapidity with which toxins are able to induce damage, that damage can be mediated by a small amount of toxin and/or that the native toxin is not highly immunogenic, each of which could preclude the development of a protective antibody response to the toxin. Hence, recovery from diphtheria or tetanus may involve different immune mechanisms and/or an immune response to different determinants than those elicited by toxoid vaccination. Although a complete understanding of the immune response to natural infection with *C. diphtheriae* or *C. tetani* would not necessarily have predicted the usefulness of toxoid vaccines, the recognition that toxins are virulence factors that mediate host damage might have identified them as potentially useful vaccine antigens. The success of toxoids as vaccines suggests that useful vaccine antigens are most likely to be discovered through the study of fundamental immune mechanisms in con-

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cert with microbial pathogenesis and the host response to microbial infection.

Live attenuated viral vaccines provide another illustration of how vaccine-elicited immunity differs from that which is elicited by natural infection. The control and clearance of viral infections is primarily mediated by cellular immune mechanisms as evidenced by the relative paucity of severe viral diseases in individual with defective B cell function. Nonetheless, the efficacy of live attenuated viral vaccines is probably a function of their ability to induce antibody-mediated immunity in the form of specific antibodies that neutralize the relevant virus (2). In natural viral infections, the appearance of specific serum antibody is a hallmark of recovery, which led to the use of antibody responses to fulfill Koch's postulate as 'immunological proof of causation' (3). However, the role that such antibodies play in host defense against natural viral infection is uncertain, as specific viral IgG often appears after control of disease and clinical improvement. On the other hand, naturally acquired antibodies can be important in the initial control of viral infection, although these antibodies are IgM, low affinity, and can be cross-reactive (4). As specific antibody responses to viruses often develop after viral clearance is underway and are long lived, they may be protective against self damage that can be mediated by a primed cellular immune system if the virus is subsequently reencountered. Thus, live viral vaccines stimulate 'unnatural' immunity in that they induce specific antibodies that are unlikely to be involved in the control/clearance of natural viral infections. The efficacy of live attenuated viral vaccines may stem from their capacity to induce a type of immune response that modulates the degree and severity of host damage resulting from the inflammatory/immune response to viral infection. Therefore, vaccine-elicited 'unnatural' immunity may counteract the host damage resulting from host-virus interaction, underscoring that the development of more effective, rationally designed vaccines is most likely to succeed if it is based on an understanding of host-microbe interaction.

Fungal diseases are an increasingly important medical problem which has been compounded by the availability of only a few effective antifungal agents and the relative inefficacy of these agents in hosts with impaired immunity (5). For example, cryptococcosis, histoplasmosis, and coccidioidomycosis are each essentially incurable in severely immunocompromised individuals, unless the immune disorder can be reversed. Although fungal vaccines would be very useful in view of the difficulty in treating invasive fungal diseases, none are currently available (6). Moreover, fungal vaccine development has been plagued by the concern that the individuals who are most likely to develop invasive fungal diseases manifest immune impairments that may preclude the generation of protective vaccine responses. There is a large body of evidence suggesting that most vaccines developed for immunocompetent hosts are relatively ineffective in hosts with impaired immunity (7). The report by Wüthrich et al. in this issue shows that it is possible to elicit vaccine-mediated protection against two fungal pathogens

in mice with CD4⁺ T cell lymphocyte deficiency, and that the induction and maintenance of protective immunity was conferred by CD8⁺ T lymphocytes (8). Detailed immunological studies showing that CD4⁺ T lymphocytes are critical components of successful host defense against both *Blas-tomyces dermatitides* and *Histoplasma capsulatum* might have predicted that vaccination with a live attenuated *B. dermatitides* strain or live *H. capsulatum* would be ineffective in the setting of CD4⁺ T lymphocyte deficiency. Surprisingly, CD4⁺ T cells were found to be dispensable for vaccine-induced immunity to these fungi. In contrast, CD8⁺ T cells were conclusively shown to mediate protection in the absence of CD4⁺ T lymphocytes.

The study by Wüthrich et al. (8) provides a new paradigm for understanding how protective immunity to fungal pathogens can be elicited, and challenges the concept that CD4⁺ T cells are required to generate the Th1 type milieu that is thought to mediate protection against *B. dermatitidis* and *H. capsulatum*. This report reveals that mechanisms of protection against these fungi are unexpectedly redundant, in that CD8⁺ T cells can induce and maintain protective immunity in the absence of CD4⁺ lymphocytes. Hence, the protection elicited by these vaccines may provide another example of 'unnatural' immunity, as CD4⁺ T lymphocytes are thought to be necessary for protection against natural infection with these fungi based on the increased incidence of these and other fungal diseases in the setting of HIV infection. On the other hand, the role that CD8⁺ T lymphocytes play in immunity in CD4⁺ T lymphocyte sufficient mice is unknown, and it is conceivable that this cell population also participates in the immune response to *B. dermatitidis* in the normal host (9). Regardless of whether the immunity elicited was 'natural' or 'unnatural', the finding that protection against fungi can be induced by different T cell populations, each of which can stimulate a Th1-like milieu, is a powerful and important result that provides support for the development of live attenuated fungal vaccines for hosts with defective CD4⁺ T cell immunity.

The observation that both the induction and maintenance of immunity to *B. dermatitidis* and *H. capsulatum* can be induced by CD8⁺ T cell lymphocytes in CD4⁺ T cell lymphocyte deficient hosts is consistent with a recent report that CD8⁺ effector T cells can be generated by CD4-independent mechanisms (10). The ability of CD8⁺ T cells to mediate protection and microbial clearance is not without precedent. For example, CD4⁺ T lymphocytes are dispensable for the induction of immunity to *Listeria monocytogenes* (11). However, recent reports have shown that CD4⁺ T lymphocytes are required to generate a CD8⁺ T lymphocyte-mediated recall response to lymphocytic choriomeningitis virus (LCMV; reference 12), and that CD4⁺ T lymphocytes, while not required for protection against primary infection with *L. monocytogenes*, are required for resistance to reinfection (11). Hence, the finding of Wüthrich et al. (8) that CD8⁺ T lymphocytes can mediate long lasting protection against fungi in the absence of CD4⁺ T lymphocytes is highly significant, with important implications for vaccination of immunocompromised hosts. The

differing requirement for CD4⁺ T lymphocytes in the generation of long lasting immunity to different microbes may reflect microbe- or host-specific factors, or factors specific to the relevant host-microbe interaction. Vaccines that stimulate CD8⁺ T lymphocyte-mediated immunity could hold great promise for protecting HIV-infected and other individuals with CD4⁺ T lymphocyte defects against fungal and other infectious diseases. In view of this possibility, the mechanism by which CD8⁺ T lymphocytes induce and mediate long term protection against some, but not other microbes deserves further study. Interestingly, in the study by Wüthrich et al. (8), unvaccinated, *B. dermatitidis*-infected wild-type mice had a greater number of lung CD8⁺ T than CD4⁺ T lymphocytes producing TNF- α and IFN- γ on days 6, 8, and 12 after infection, a pattern which was reversed in wild-type mice that were vaccinated with the live attenuated *B. dermatitidis* vaccine. Therefore, CD8⁺ T lymphocytes may be involved in the 'natural' immune response to wild-type *B. dermatitidis* in CD4⁺ T lymphocyte sufficient mice, albeit possibly to their detriment, since they succumb to disease.

An interesting mechanism to explain vaccine-mediated protection against *B. dermatitidis* and *H. capsulatum* is antibody-mediated immunity. On one hand, there is little evidence that antibody-mediated immunity makes a significant contribution to host defense against these fungi, as evidenced by the fact that the incidence of invasive fungal disease is not increased in patients with antibody deficiency, the inability to demonstrate efficacy in passive protection experiments and the finding that B cell-deficient mice are not more susceptible to experimental (natural) infection than normal mice. On the other hand, an increased incidence of invasive fungal diseases such as cryptococcosis, histoplasmosis, and coccidiomycosis has been noted in patients with combined B and T cell deficiency, such as individuals with HIV infection and organ and bone marrow transplant recipients receiving immunosuppressive therapies. Notably, it has been shown that although B cells are not required for the induction of protective immunity to *L. monocytogenes*, they prevent contraction of antigen-specific CD8⁺ T lymphocytes (13). Therefore, B cells could modulate the availability of effector T cells, a role that would be consistent with the concept that antibodies and/or B cells can regulate the outcome of cell-mediated immune responses. Along these lines, B cell-deficient mice are unable to resist reinfection with certain fungi, despite their resistance to primary infection and the ability to generate activated Th1-type cells (14). However, opsonizing antifungal antibodies are able to reconstitute the ability of dendritic cells to secrete key cytokines involved in resistance to invasive fungal disease in the setting of B cell deficiency (14). These findings underscore the interplay between antibody and cell-mediated immune mechanisms in mediating protection against certain fungi. An important outcome of this interplay may be a reduction in the host damage resulting from the host-microbe interaction. In this regard, it is noteworthy that in the report by Wüthrich et al. (8) vaccine-mediated protection was accompanied by

reduced inflammation (granuloma formation) and fungal burden in the lung, although mice lacking both CD4⁺ and CD8⁺ T lymphocytes were also able to control the fungal burden despite the presence of inflamed granulomas. Hence, the possibility that antibodies and/or B cells, in addition to other cell types, such as dendritic or NK cells, may play a role in the development of, or in the effector phase of antifungal immunity, has implications for vaccine development and deserves further study.

Live attenuated vaccines were responsible for the control of smallpox, polio, measles, and mumps. These vaccines are highly immunogenic, but have been thought to be associated with a significant risk of disease due to the attenuated vaccine strain in immunocompromised patients. Consequently, the use of live vaccines is usually contraindicated in hosts with impaired immune function. However, there are notable exceptions, most importantly the recommendation for and use of the measles-mumps-rubella (MMR) vaccine in HIV-infected children, and a live attenuated varicella zoster vaccine in immunocompromised children. In recent years, the success of immunization with component vaccines such as recombinant Hepatitis B surface antigen vaccines and *Haemophilus influenzae* and *Streptococcus pneumoniae* polysaccharide-protein conjugate vaccines, and concerns about vaccine-induced disease in compromised hosts have fueled a trend away from research into live vaccines. However, component vaccines elicit significantly weaker immune responses in immunocompromised individuals, in whom such preparations may be safe but are also relatively ineffective (7). In light of the above noted successful precedents for the use of live attenuated vaccines in immunocompromised patients, the encouraging preclinical data reported by Wüthrich et al. (8) should bolster further consideration of the development of live attenuated vaccines. Live attenuated vaccines may ultimately be more effective than component vaccines in immunocompromised patients, particularly if they are able to direct the immune response toward the use of available cellular and/or antibody subsets. In this regard, the live attenuated *B. dermatitidis* vaccine used in the study of Wüthrich et al. was generated by deleting an adhesin, which is both an important virulence factor and an immunodominant antigen that can elicit a protective immune response (9). It is noteworthy that this attenuated strain was able to induce immunity to a wild-type strain that expresses the adhesin. Therefore, an immune response to the adhesin is not required for vaccine-mediated protection against the wild-type strain, suggesting that the response to other determinants is important to produce immunity, which may in part account for why antibodies to this determinant were not found to be protective in a previous study (15). The development of the *B. dermatitidis* vaccine strain illustrates how the use of the recombinant DNA technology and gene manipulation to discover mutant microbial strains with attenuated virulence can identify promising vaccine candidates. However, the exciting finding that CD4⁺ T lymphocyte-deficient hosts were successfully vaccinated against lethal fungal diseases has not necessarily narrowed the gap between vaccinology

and immunology. Convergence of the two fields will require a more complete understanding of immunology and microbial pathogenesis, such that the former becomes a predictive discipline where the efficacy of antigens as vaccines can be predicted from first principles. Until this occurs the gap between vaccinology and immunology may be both necessary and desirable. The vaccination studies described by Wüthrich et al. (8) illustrate the synergism that is possible when the empiricism often associated with vaccinology is combined with the mechanistic/regulatory studies championed by immunology.

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