Immunogenicity may also be enhanced by altering protein presentation on the surface of infected cells. Fusion of a secreted malaria blood stage antigen to the murine immunoglobulin G transmembrane anchor sequence resulted in surface expression of the protein and a greatly enhanced antibody response (95). A related strategy has been employed in which repeating epitopes of the malarial circumsporozoite protein (CSP) were fused to the ectodomains of the RSV glycoprotein G, thereby enhancing anti-CSP antibody titers in vaccinated animals (96). Elimination of proteolytic cleavage sites from the HIV env gene prevented release of gp120 from the env precursor gp160, and the resulting recombinant produced a stronger anti-gp120 antibody response in vaccinated animals, presumably by increasing the amount of surface-associated gp120 (97).

The incorporation of additional helper T-cell epitopes in expressed antigens may also increase immunogenicity through enhanced recruitment and proliferation of B cells. A short peptide derived from the sequence of the neutralizing epitope of the viral capsid protein VP1 of foot-and-mouth disease virus produced a weak antibody response in cattle and pigs. However, fusion of the VP1 epitope to Hepatitis B core protein (HBcAg) led to a dramatic increase in antibody titers in animals vaccinated with the purified protein expressed by recombinant vaccinia virus and greatly enhanced virus neutralization (98). Inclusion of murine helper T-cell epitopes led to increased titers of anti-malarial antibodies in mice vaccinated with a CSP fusion construct (99).

Protein targeting may also enhance immunogenicity. Fusion of antigen genes to endoplasmic reticulum or lysosomal targeting sequences may direct expressed proteins into intracellular compartments where processing for antigenic recognition is facilitated, boosting the immune response to viral and tumor-associated antigens. In one case, endoplasmic reticulum targeting was reported to increase $CD8^+$ T cell recognition of tumor cells (91). Fusion of HIV gp160 sequences to the lysosomal targeting sequence LAMP-1 boosted the $CD4^+$, class II-restricted effector T-cell response to the expressed peptide, through enhanced transport of peptide into processing compartments (100). In addition, boosted antibody titers, lymphoproliferative, and $CD4^+$ CTL responses followed immunization with a vaccinia LAMP-1/human papillomavirus E7 construct, as compared to the standard construct (101).

Recombinant poxviruses that co-express certain cytokines or other mediators exhibit increased immune responses. Expression of interleukin (IL)-2 enhanced the serum IgG response to influenza nucleoprotein (102), and expression of IL-5 or 6 increased the secretory IgA response to influenza HA following intranasal vaccination (103). IL-2 co-expression with β-galactosidase enhanced activity against β-galactosidase-expressing tumors in mice (104). Similarly, expression of granulocyte/macrophage colony-stimulating factor produced by recombinant avian poxviruses enriched the regional lymph nodes with antigen-presenting cells and acted as an immunoadjuvant (105). Expression of the CD28 ligand B7 by recombinant vaccinia viruses enhanced antitumor activity in mice by mediating a Th1-type T-cell response (106). Synergistic effects were reported for a triad of costimulatory molecules namely, B7-1, ICAM-1, and LFA-3 (107). Interferon-γ, IL-12, IL-21 have also been reported to provide enhanced immune responses (108–110).

Heterologous priming and recombinant poxvirus boosting greatly enhanced T-cell responses. This was first noted using a recombinant influenza virus as the prime followed by a recombinant vaccinia virus (111). This approach works particularly well using a DNA prime followed by a vaccinia virus or avipoxvirus boost (112–114). The generally accepted explanation is that immune competition between the many poxvirus proteins and the recombinant protein limits the extent of the immune response to the latter. However, if the animal has already made a primary immune response to the recombinant protein, then the recombinant vaccinia virus can preferentially boost this.

The presence of maternal antibodies is a major obstacle to vaccination of infants with some attenuated viruses, such as measles, and also diminishes the immune response to recombinant vaccines. Passive administration of antiserum to influenza A (115) or RSV (116,117) abrogated both the desired antibody response and disease protection mediated by vaccinia-based influenza or RSV vaccines. Replication of the recombinant viruses and stimulation of antibodies to vaccinia proteins was unaffected, indicating that antibodies to the influenza or RSV proteins produced specific immune interference. Normal immune responses were restored after waiting for clearance of passively administered antibodies to measles virus in mice repeatedly vaccinated with vaccinia/measles recombinants (118). The inhibitory effect depends on the level of passive antibody, however, and a recombinant vaccinia virus provided significant protection of monkeys against measles infection (119). Changing the site of inoculation may partially overcome passive blockade of antigen, since intranasal administration of vaccinia/RSV vectors was significantly more immunogenic than dermal administration in cotton rats given anti-RSV antiserum parenterally (120).

Existing immunity to the vector is a potential problem with any live recombinant virus, and this also holds true for vaccinia virus (71,121). Because of the cessation of smallpox vaccination in the early 1970s, individuals under 35 are generally vaccinia naive. Systemic immunity to vaccinia virus can be circumvented to some extent by administering the recombinant vaccine by a mucosal route (122). Repeated vaccination or priming with a DNA vaccine may also be useful. Because of their large genetic differences, immunity to vaccinia does not appreciably affect vaccination with avipox vectors.

Safety

During the extensive use of vaccinia virus as a smallpox vaccine, adverse reactions in addition to the routine swelling and soreness at the site of administration and low-grade fever were observed. The most serious of these were progressive or disseminated infection in immunocompromised individuals, eczema vaccinatum, and postvaccinal encephalitis or encephalopathy in infants. The incidence of the latter was reported to vary with different vaccine strains, ranging from 1 in 2000 for the Copenhagen strain, to 1 in 200,000 or more for the New York City Board of Health (Wyeth) and Lister strains (1,123). There is a fear that adverse reactions would be even more prevalent now because of the high incidence of HIV, use of immunosuppressive drugs in transplant patients, and increased atopic dermatitis.

Prior to the eradication of smallpox, more attenuated strains of vaccinia virus were made by serial passage in tissue culture (1). MVA, the attenuated strain most extensively tested in humans, was passed 570 times in cultured chick embryo cells and induces only a slight reddening at the site of inoculation (124,125). MVA has many gene deletions (126,127) resulting in