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MINIREVIEW

Cytokines as Adjuvants for Vaccines: Antigen-Specific Responses Differ from Polyclonal Responses

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INTRODUCTION

Since infections caused by a wide variety of microorganisms are responsible for considerable mortality and morbidity worldwide, efforts to develop strategies to inhibit the growth of the specific causative agent and ultimately reduce the spread of such infections have received much attention. Among these strategies, the development of vaccines is a major priority; here, it is important that the appropriate immune response is generated. For instance, in the development of vaccines for diseases in which humoral immunity is the most effective means of protection, it is not only essential to obtain adequate amounts of antibody but also important to generate a particular immunoglobulin isotype in order to ensure that the causative agent is effectively eliminated. Certain immunoglobulin isotypes are more efficient than others in activating complement or in binding to receptors on monocytes. Thus, it is often desirable to employ adjuvants that will induce the synthesis of isotypes with greater protective value. In general, the primary objective of increasing the antibody response per se is achieved by employing chemical adjuvants to boost the response. However, because of the need for obtaining antibody of an appropriate isotype, it is not surprising that the use of recombinant cytokines, rather than standard formulations, as adjuvants for vaccines is getting considerable attention. Cytokines have been shown to be involved in many aspects of the immune response. These include the activation, proliferation, and differentiation of B and T lymphocytes; the activation and expansion of natural killer cells and cytotoxic T cells; the activation of mononuclear phagocytes; and the recruitment of immunocompetent cells to the sites of infection (12, 38, 40).

The majority of these observations have now been supported by the use of cytokine-deficient mice made by gene targeting (25, 26, 38). Such studies have emphasized the view that cytokines are redundant (i.e., different cytokines perform the same function) and pleiotropic (i.e., cytokines act on many different cell types) in their activities (12, 38, 40). Certainly, one of the most attractive findings related to cytokine immunobiology is the observation that the synthesis of a particular antibody isotype produced is influenced by specific cytokines. Certain cytokines have the capacity to increase the formation of a particular antibody isotype without significantly influencing the quantity of the others; for example, interleukin-5 (IL-5) or transforming growth factor β can augment immunoglobulin A (IgA) antibody formation (12, 29), whereas gamma interferon (IFN-γ) can increase the synthesis of IgG2a antibody (6, 8, 9, 12, 13, 42). In addition, IL-4 is able to induce switching to the IgE isotype (12, 13, 15). However, most of these initial findings were based on the analysis of polyclonal or non-antigen-specific rather than antigen-specific antibody responses. The intent of this review is to focus attention on this issue and discuss the significance of such observations with regard to vaccine development, in which the generation of antibody of a given isotype is of utmost importance.

IMMUNOBIOLOGICAL EFFECTS OF CYTOKINES: ANTIGEN-SPECIFIC VERSUS POLYCLONAL RESPONSES

Over the last decade, and in particular during the last few years, a number of studies (10, 11, 19, 36, 46) have indicated that the effects of cytokines depend on whether one examines an antigen-specific or a nonspecific (polyclonal) response (Table 1). For example, the in vivo administration of IL-4 for a period of four months caused an increase in the levels of serum polyclonal IgG1 and IgE with no change in the level of serum polyclonal IgG2a (46). By contrast, in the same study, there was a marked decrease in the levels of antigen-specific (trinitrophenyl [TNP]-keyhole limpet hemocyanin [KLH]) IgG1 and IgE levels and the formation of memory B cells was decreased in the same animals (46) (Table 1). One possible explanation for these diverse effects is that the administration of IL-4 leads to an imbalance of other cytokines (e.g., IFN-γ) in vivo and that this would have a different influence on antigen-specific and polyclonal responses. In another study, it was found that antigen-specific IgE production following antigen (TNP-KLH) stimulation is not altered by IFN-γ, whereas the generation of a polyclonal IgE response induced by lipopolysaccharide (LPS) from gram-negative bacteria is suppressed by IFN-γ (36) (Table 1). Although it is conceivable that additional cytokines may be needed to alter the antigen-specific response, it is possible also that B cells generated during the course of an antigen-specific response differ from B cells involved in polyclonal responses in their sensitivity to IFN-γ. Differences between antigen-specific and polyclonal responses also have been noted in in vitro studies of the role of IL-4; here, in the presence of monoclonal anti-IL-4 antibody, antigen-specific (TNP-conalbumin) IgE synthesis was not abolished, nor was the antigen-specific IgE antibody enhanced by the addition of recombinant IL-4 (19). By contrast, the polyclonal IgE response was abolished by treatment with anti-IL-4 antibody (19). These studies also show that the antigen-specific IgE response is not affected even in the presence of anti-IL-4 antibody and antisense oligonucleotides (i.e., specific for IL-4 mRNA) (19). This suggests that the generation of polyclonal IgE, unlike the antigen-specific antibody response, requires IL-4.

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TABLE 1. Effects of various cytokines on antigen-specific and polyclonal responses

Cytokine or blocking antibody	Antigen-specific response		Polyclonal response		Reference
	Isotype(s) measured	Effect[a]	Isotype(s) measured	Effect[a]	
IL-4	IgE, IgG1	↓	IgE, IgG1	↑	46
			IgG2a	↔	
IFN-γ	IgE	↔	IgE	↓	36
Anti-IL-4	IgE	↔	IgE	↓	19
Anti-IL-4	IgM, IgG1, IgG2a, IgG2b, IgG3	↓	IgM	↑	10
			IgG1, IgG2a, IgG2b, IgG3	↔	
IL-10	IgM, IgG1, IgG2a, IgG2b, IgG3	↓	IgM, IgG2a, IgG2b, IgG3	↑	10
			IgG1	↓	

[a] ↑, increase; ↓, decrease; ↔, no change.

Studies conducted in our laboratory indicate that the administration of anti-IL-4 antibody prior to immunization with *Pseudomonas aeruginosa* LPS (PALPS) results in a decreased PALPS-specific antibody response for IgM and all IgG isotypes (IgG1, IgG2a, IgG2b, and IgG3) examined (10). By contrast, the polyclonal IgM response is enhanced whereas the other IgG isotypes remain unaffected. Also, these studies (10) show that when mice are given recombinant IL-10 at the time of immunization with PALPS, there is a decrease in the PALPS-specific antibody response for all isotypes examined; however, polyclonal IgM, IgG2a, IgG2b, and IgG3 responses are increased, whereas the polyclonal IgG1 response is decreased (Table 1). The results of our studies, which are consistent with those mentioned above, indicate that antigen-specific and polyclonal responses are influenced in a different manner by cytokines. Although the role of cytokines other than IL-4 or IFN-γ has not been well documented, studies of the effects of IL-6 on both antigen-specific and polyclonal responses also reveal considerable differences (7, 47).

Besides the direct effects of cytokines on antigen-specific and polyclonal responses, several observations have been made using agents capable of inducing cytokines. For instance, in one study, treatment with complete Freund's adjuvant resulted in a large increase in polyclonal IgG2a but no significant change in the antigen-specific IgG2a antibody response (5). In view of studies conducted by other investigators (6, 8, 11, 12, 42), it is possible that the increased formation of IgG2a is mediated by INF-γ, which is presumably induced by complete Freund's adjuvant. These observations underscore the fact that both antigen-specific and polyclonal responses must be examined before an appropriate adjuvant is selected for use in a vaccine. Two other studies using two different chemical compounds also indicate differences between both types of responses. First, the administration of D-penicillamine (DP), an immunosuppressive thiol compound, decreases the antibody response to tetanus toxoid (39). By contrast, the compound has no significant effect on Epstein Barr virus-induced polyclonal immunoglobulin production. It is known that DP has immunosuppressive properties; however, it is not clear whether cytokine synthesis is altered after the administration of DP. Such compounds may be useful in identifying differences between polyclonal and antigen-specific responses and defining the mechanisms by which both responses differ. Second, astaxanthin (a carotenoid) has the capacity to increase the antigen-specific response to a T-cell-dependent antigen, without altering the response to a T-cell-independent antigen or the generation of a polyclonal response (21). Although mice that have been given astaxanthin produce high levels of IL-2 in response to polyclonal stimulation, relatively low levels of IL-2 are induced following antigen-specific stimulation. Thus, the inability to augment a polyclonal response cannot be explained by the lack of IL-2 production.

It has been established that age can affect the magnitude and type of the immune response generated. For example, in aged mice there are differences in the ability of T cells from the same lymphoid organ to respond to antigen-specific versus polyclonal responses (23). In addition, these studies (23) also show that whereas lymph node T cells from aged mice produce high amounts of IL-2 in response to polyclonal stimulation (i.e., using the mitogen concanavalin A), very little IL-2 (or INF-γ) is made in response to antigen-specific stimulation. Although this particular study shows that the immune response depends on other variables, including the genetic background and the source of lymphocytes, it supports the underlying theme of the other studies, namely that aging, which by itself alters the ability to make as well as respond to cytokines, has differential effects on polyclonal and antigen-specific responses.

Perhaps the most relevant findings on differences between polyclonal and antigen-specific responses are those obtained using peripheral blood mononuclear cells from patients with AIDS. They show that although the capacity of B cells to make an antibody response to sheep erythrocytes is severely depressed, the ability to respond to polyclonal agents is unaffected (22). The results of previous studies carried out by these investigators also indicate that the antigen-specific response to a different antigen, KLH, also is depressed (28). It is possible that the inability to generate optimal antigen-specific responses may be due to a number of factors; these include the lack of adequate amounts of cytokines for the antigen-specific B cells, the lack of expression of appropriate cytokine receptors or receptors for cellular adhesion molecules, or the inability to effectively transmit signal transduction events. In the light of the differences observed in the studies described thus far, the question arises whether elevating the cytokine dose would change the magnitude of the antigen-specific response. Such questions should be addressed in future studies.

In this review, no attempt has been made to discuss events in which combinations of cytokines are used in an effort to obtain synergistic or counteractive effects. It should be noted also that vaccines targeted against some of the organisms mentioned in this review, such as *P. aeruginosa* or *Streptococcus pneumoniae*, also elicit specific antibody responses. In this case, as with that of laboratory studies mentioned in the previous paragraphs, it is assumed that the antibodies generated are protective. Indeed, the studies mentioned above indicate differences between antigen-specific and polyclonal responses and raise a number of important questions. Are separate subsets of B cells involved in producing polyclonal and antigen-specific responses, or is the same B-cell population being activated via

different signalling pathways following a polyclonal or an antigen-specific stimulus? Do cytokines have a direct effect on B cells with respect to both types of responses, or are these effects mediated by other cell types?

THE EXISTENCE OF B-CELL SUBPOPULATIONS

It is possible that the antigen-specific and polyclonal responses may differ with respect to unique biochemical events intrinsic to B cells. Studies indicate that two distinct populations of B cells may be involved in antigen-specific and polyclonal responses (4, 20, 35, 45). One involves populations of B cells ($CD5^-$) that are adequately represented in the spleen and lymph nodes of adult animals. A second population ($CD5^+$, also known as B-1 cells) predominate early in life and produce the so-called natural IgM antibody responses, characteristic of older mice and those with autoimmune diseases, and generally respond to polyclonal stimulation. Indeed, a number of investigators have identified similar subsets in humans (4, 16, 45). It has been reported that B cells responding to antigen-specific responses are $CD5^-$; for example, the ability to make antibody to the capsular polysaccharide of type IV *S. pneumoniae* is restricted to the $CD5^-$ population (4); this is consistent with the observation that neonates respond poorly to bacterial polysaccharide antigens (2, 3, 37). Furthermore, it has been shown that the generation of anti-human immunodeficiency virus-specific antibody is confined to the $CD5^-$ B-cell subset (20), and interestingly, seropositive individuals with limited or advanced disease show a large increase in the $CD5^+$ subset (i.e., those B cells capable of responding to polyclonal stimulation). Although it has been shown that cytokines produced by polyclonally induced T cells are different from those made by antigen-stimulated T cells (18), it is not clear whether the cytokine requirements for antibody production by $CD5^-$ B cells differ from those of the $CD5^+$ B-cell population.

Little is known about the molecular mechanisms by which cytokines influence antigen-specific or polyclonal responses. Studies suggest that there are different signalling pathways involved in eliciting an antigen-specific or a polyclonal response (24, 27, 44, 48). However, more information is needed to clarify how both systems differ in the initial signal transduction events as well as in the generation of specific immunoglobulin subclasses.

PRACTICAL CONSIDERATIONS

Because a number of subunit vaccines are poorly immunogenic, the addition of cytokines to the standard adjuvant formulations is being given serious consideration not only for humans but also for domestic animals. Several studies show that the administration of cytokines boosts both the humoral and the cellular immune responses greatly (14, 17, 30–34, 42, 43). Some of these studies indicate that cytokines improve the ability of a vaccine to confer protective immunity in disease models. For example, studies show that immunization with herpes simplex virus glycoprotein–IL-2 complex is effective against the disease in guinea pigs (32). Here, the efficacy of IL-2 as an adjuvant can be further improved if the cytokine is encapsulated into liposomes. It has also been shown that IFN-γ increases the therapeutic effects of chloroquine in malaria (14); here, the survival rate is correlated with increased levels of IgG2a antibodies. In a recent study, it was shown that IL-12 has an adjuvant effect in a vaccine against *Leishmania major* (1). In this study (1), the vaccination of normally susceptible BALB/c mice with leishmanial antigens in the presence of IL-12 shifted the cytokine pattern from a Th2 type to a Th1 type, resulting in greater control of infection (1). Manipulation of the cytokine response in this way represents an important advance in cytokine research. Because other studies have shown that a number of bacterial products can stimulate IL-12 production, it is conceivable that bacterial adjuvants may function by elaboration of this cytokine.

Although most of the issues raised in this review have been targeted towards antigen-specific responses, this effort should not neglect the significance of persistent polyclonal stimulation (14, 16, 41). Indeed, there are a number of viral, bacterial, and parasitic diseases in which a major concern is dealing with the undesirable effects of polyclonal stimulation. In the case of parasitic infections, the increase in immunoglobulin formation, which follows extensive polyclonal stimulation, may lead to immune complex formation and subsequent deposition in the kidneys, with severe immunopathological reactions. During malaria infections, differences between polyclonal and antigen-specific responses have been observed in the time course of the antibody response (41); maximum antibody responses to sheep erythrocytes are observed early during the course of malaria infection, whereas maximum polyclonal responses are observed 2 to 3 weeks later. Consequently, attempts to ameliorate any undesirable effects of polyclonal stimulation must take into account these differences in the time course of both types of responses, particularly when vaccines are administered to populations living in areas endemic for a variety of chronic infectious diseases.

Indeed, cytokines play a major role in the regulation of immune responsiveness. For instance, during the immune response to mycobacteria, cytokines such as IL-10, IL-13, and transforming growth factor β can suppress the immune response whereas proinflamatory cytokines such as IL-1, IL-6, and tumor necrosis factor can augment the response, thereby regulating the development of cell-mediated immune responses (49). Therefore, the administration of cytokines as adjuvants can be viewed as upsetting the balance of cytokines in the host. Thus, in some cases, the use of specific cytokine antagonists may have more therapeutic value. In addition, because of the complexity of cytokine requirements for antibody production, it is likely that a combination of cytokines may be more effective than the administration of single cytokines.

SUMMARY

The use of cytokines in the administration of vaccines has a unique value in obtaining the appropriate immune response and in ensuring a protective outcome. Earlier studies indicating that cytokines can influence the generation of a particular antibody isotype may represent an oversimplification of a more complex problem. Several studies discussed in this review show that the effect of a given cytokine on the immune response depends on whether one examines the antigen-specific response or the polyclonal response (i.e., total serum immunoglobulins). Further, a balanced regulation of immune responsiveness is important in maintaining homeostasis of the immune system. Consequently, for any vaccine that uses cytokines to boost the response, due consideration must be given to these important variables.

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