INFECTION AND IMMUNITY, Nov. 1988, p. 2769–2773
0019-9567/88/112769-05$02.00/0
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Vol. 56, No. 11

MINIREVIEW

Immunization against Tuberculosis: What Kind of Vaccine?

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INTRODUCTION

Contrary to popular belief, tuberculosis is a severe health problem (30, 31, 36) that is getting worse rather than better (27, 30, 31, 36, 66). It is perpetuated and exacerbated by poor living conditions, rising bacillary resistance to multiple antituberculosis drugs, acquired immune deficiency syndrome, and especially lack of a suitable vaccine. Recent international conferences convened to analyze this urgent problem (2, 65a, 74) reached two important conclusions about antituberculosis immunization. One was that it be given highest priority in a renewed attack on the disease, and the other was that the currently used vaccine, *Mycobacterium bovis* BCG, must be replaced by something considerably better and safer (see also reference 64). BCG has not proved usefully effective in developing countries, where immunization is most needed. Furthermore, as a living, attenuated tubercle bacillus, it is dangerous to anyone with human immunodeficiency virus infection, in whom it can cause a progressive, fatal bacillemia (32, 36, 55, 70). Thus, after a hiatus lasting a full generation, basic research aimed toward better antituberculosis immunization is once again being done (31, 59).

Radically new vaccines are being made possible by gene cloning techniques, modern molecular biology, and greatly improved understanding of cellular immunology (31). Most attractive for a new antituberculosis vaccine would be a Trojan horse vector (37; see also reference 63). A gene for the immunizing antigen would be cloned and put into a virus or bacterial vector. Then, the vector injected into a subject would produce immunizing antigen and give it the adjuvanticity it needs to immunize (37). Work in this direction has begun. Genes for certain proteins of tubercle bacilli have in fact been cloned and expressed in foreign microbial vectors (e.g., references 9 and 46; see also reference 37).

However, there appears to be an important, generally unrecognized obstacle to developing this kind of vaccine: the immunizing epitope of tubercle bacilli probably is a polysaccharide. Most evidence for this is relatively old. Many of the original papers providing it are not known or readily accessible to new investigators in tuberculoimmunity. The purpose of the present review therefore is to recall and concisely review knowledge of the chemistry of the immunizing antigen(s) and from that to speculate on how a new vaccine might best be developed.

NATURE OF TUBERCULOIMMUNITY

Tuberculoimmunity is cell mediated (5, 18, 20, 33, 77 [chapter 8]). Serum antibodies (humoral immunity) have no protective role in it (56, 57). Presumably, it is expressed by phagocytic monocytes responding at the site of infection to appropriate activation by immune lymphokine(s). An immune lymphokine is probably made by T lymphocytes specifically reacting with the immunizing antigen of the tubercle bacillus. A particular immunizing antigen must exist, for there are many data showing that most of the antigens of tubercle bacilli are not protective, even though many can induce and elicit vigorous cell-mediated (hypersensitivity) reactions (see below). A special antigen is further suggested by recent data showing that lymphokines such as gamma interferon and tumor necrosis factor, which are produced abundantly by cell-mediated reactions to the nonprotective (sensitizing) antigens, are responsible more for the pathology than the immunity of tuberculosis (18, 59, 60, 75).

Why a particular antigen is needed for induction and expression of tuberculoimmunity is not yet understood. It may be necessary for stimulating a subset of T cells dedicated to making the (still unidentified) molecular message of immunity which is needed to appropriately activate human macrophages so that they can inhibit tubercle bacilli (see reference 47).

IMMUNIZING ANTIGEN

By definition, the immunizing antigen has to be able to induce protective immunity. Antigens that induce cell-mediated immunologic responses which are not protective or, on the contrary, are tissue-destructive hypersensitivities are not logical candidates for an immunizing vaccine.

The existence of an immunizing antigen in tubercle bacilli has been seriously questioned because dead bacilli or extracted antigens have frequently been unprotective. It has been thought that living bacilli (e.g., BCG) are required and that the immunity is induced by a dynamic living bacteria-host interaction. This idea is still being used to explain certain experimental results (50). But evidence that dead bacilli and extracts of the bacilli are able to immunize, and therefore that there is an extractable immunizing antigen, is very strong (see below).

Several methods for obtaining immunizing antigen have, in fact, been devised. By the time most research on immunizing antigen ceased, nearly two decades ago, there was good information about which kinds of antigen are protective and which are not. This information was last definitively reviewed in 1958 (10) and 1959 (73). It has also been reviewed more recently (16, 41, 49, 65, 77 [chapters 10 and 11]), incidental to other subjects. The following is a condensed review of old and new information on this topic.

ADJUVANTICITY

Adjuvanticity, essential to immunization against tuberculosis, is usually unrecognized. Lost adjuvanticity probably is the main cause for poor immunization by nonliving bacilli or

by antigens extracted from them. Empirically, this adjuvanticity can be provided by a properly formulated incomplete Freund adjuvant. In this, killed bacilli and certain extracts of them regularly induce immunity as good as can be induced with living bacilli (16).

While the mechanisms of adjuvanticity are still only dimly understood, a long series of experiments with cell-mediated hypersensitivity to the proteins of tubercle bacilli has clearly demonstrated its indispensability for inducing cell-mediated immunologic responses (see references 57 and 75). The findings were, in fact, parallel to what has been observed with extracted antigens or killed bacilli in induction of tuberculoimmunity. Thus, living bacilli readily induced delayed hypersensitivity to tuberculoproteins. But dead bacilli or the purified proteins alone could not unless they were injected with appropriate adjuvants, usually in some form of Freund water-in-oil emulsion. The research showed ultimately that while the proteins were antigenic (that is, were the specific targets of immunologic reaction, once this had been induced), they needed help from peptidoglycolipid adjuvants, extractable with mineral oil or similar solvents (a reason why Freund adjuvants are fortuitously so helpful for induction [7]) from the bacilli themselves, to induce a cell-mediated immunologic response. The proteins were separated from these adjuvants when they were extracted from the bacilli and purified and then were unable to sensitize unless artificially recombined with bacillus-derived or other comparable adjuvants.

These principles and problems of adjuvanticity are very important in considering how to design a new antituberculosis vaccine because they explain why killed bacilli and extracts of the bacilli are less effective than living bacilli for immunization and they indicate that to be effective the new vaccine has to combine appropriate adjuvanticity with the proper antigen. This concept of inseparability of adjuvant and antigen for induction of classic cell-mediated immunologic responses is different and distinct from concepts of what constitutes an antigen capable of inducing humoral antibody responses.

CHEMICAL NATURE OF THE IMMUNIZING ANTIGEN

In animal experiments, an incomplete Freund adjuvant in which both the oil (*n*-hexadecane) and the aqueous phase (containing phosphate ions; see references 11 and 21) were properly formulated was found empirically to provide the necessary adjuvanticity for killed bacilli and certain antigens extracted from them to induce tuberculoimmunity as effective as any inducible by living bacilli, even virulent ones (16). With this type of adjuvant, it became possible to investigate which bacillary antigens could immunize, because most of their loss of adjuvanticity by extraction and purification could be compensated for.

Results from several laboratories doing many experiments indicated that the immunizing antigen, or more precisely the immunizing epitope, is not a protein (15, 57, 77 [chapter 13]). Tuberculoproteins were at first considered the most likely candidates because they were known to be responsible for classic cell-mediated tuberculin-type delayed hypersensitivity. Moreover, in practice there usually is a correlation between development of tuberculin hypersensitivity, as detected by skin testing with purified protein derivative, and development of immunity (77 [chapter 13]). However, immunity could be induced or be present without tuberculoprotein hypersensitivity and vice versa (77 [chapter 13]). No verifiable evidence that any of the proteins can immunize has ever been produced. Tuberculoproteins were last formally and carefully tested for the potential to induce tuberculoimmunity in 1974 by Reggiardo and Middlebrook (57). These workers showed that guinea pigs strongly sensitized with the proteins, even if given supplemental humoral antiprotein antibodies, were unable to express immunity to airborne infection. The proteins instead of being protective most frequently have proved to be pathogenetic. For instance, in a three-decade series of experiments (see references 75 and 76), Yamamura et al. repeatedly have shown that the cell-mediated immunologic reaction to tuberculoproteins and related peptides is a prominent cause of the tissue destruction and cavity formation characteristic of tuberculosis.

Unlike the proteins, other kinds of antigens have frequently proved protective (49). These have included crude lipids, RNA-rich extracts (ribosomal vaccines), and especially polysaccharides (10, 12, 15, 16, 40, 43–45, 48, 52–54, 69, 71). The protective epitope now appears to be a polysaccharide in both lipids (13, 38, 39, 58) and in RNA-rich extracts (52–54). The immunizing antigen which has been most extensively studied, obtained from defatted tubercle bacilli by trypsin or similar digestion and reported in 1981 to be able to immunize human subjects (22), is a carbohydrate consisting of the sugars arabinose, mannose, and galactose. It appears to be attached to a peptide which could very well have an important carrier (adjuvant) role in its immunogenicity (16; see references 52 and 54 for more recent research on an apparently similar polysaccharide immunizing antigen extracted differently and also depending on accessory molecules for immunogenicity).

The seeming heterogeneity of the immunizing antigen (48) probably results from the protective epitope being in the bacterial cell wall (14, 15, 58), from which it is extractable in several different ways (15, 16, 35, 42).

CARBOHYDRATE ANTIGEN AND CELL-MEDIATED IMMUNOLOGIC RESPONSES

For the immunizing epitope to be a carbohydrate would appear to conflict with popular belief that only proteins can induce and elicit cell-mediated immunologic responses. There is much evidence, however, that polysaccharides can induce and elicit cell-mediated immunologic responses (e.g., references 8, 19, 51, 68, and 72), even polysaccharides of the tubercle bacillus (4, 6, 24). A large body of well-known literature on contact hypersensitivity proves independently that epitopes for cell-mediated immunologic reactions do not have to be proteins. Using polysaccharide antigens for antimicrobial immunization is in fact beginning to receive serious consideration (34).

The basic requirement for epitope involvement in cell-mediated immunologic reactions appears to be that it be available on the membrane of an antigen-presenting cell in conjunction with the HLA-DR histocompatibility antigen for syntactical recognition by T-cell paratopes. The main argument against a polysaccharide being able to induce a cell-mediated immunologic response is negative—that there is not yet any precedent for processing of a polysaccharide antigen for presentation in proper association with the membrane HLA-DR antigen. There are three prominent weaknesses to this argument. (i) Antigen processing is not a universal requirement for such antigen presentation (1). (ii) Suitable association with an antigen-presenting cell membrane may occur spontaneously (3). Tubercle bacillus antigens bind readily to mammalian cell membranes (67). (iii) The immunizing carbohydrate epitope may be associated

with an adjuvant peptide carrier whose function is to integrate the carbohydrate into the membrane of the presenting cell. While unable itself to immunize, this peptide may be essential for the polysaccharide to immunize. Reduction of immunogenicity has in fact been reported for a peptide-containing immunogenic polysaccharide treated with pronase (16). A similar adjuvant-carrier function for immunizing polysaccharide has been reported for RNA (52).

ROUTES TOWARD DEVELOPMENT OF A NEW VACCINE

If the immunizing antigen is not a protein but rather a polysaccharide, as much experimental evidence indicates, gene cloning and the Trojan horse approach, although very attractive, seem unlikely to succeed without some unanticipated advance in this kind of technology for controlling the manufacture of polysaccharide antigens in surrogate host microorganisms (31). An alternative, currently more promising approach to devising a new antituberculosis vaccine might be as follows.

The immunizing polysaccharide epitope probably can be synthesized (see reference 29). Whether it alone can be properly presented on human macrophages for cell-mediated reaction and thus immunization could be determined by intradermal skin testing of immunized human volunteers, as currently can be done with the natural antigen, which has been purified but retains some contaminating peptide (16). It could also be tested in vitro for the ability to induce T cells from immunized, but not unimmunized, subjects to respond to it on autologous monocytes or macrophages. The responses of the T cells could be measured in various ways, ranging from the rapid and easy induction of blastogenesis to the immunologically much more relevant, but also slower and more difficult, determination of the ability of the stimulated T cells to protect autologous macrophages against tubercle bacillus infection (18, 22).

Doing these experiments with animals or animal cells, for development of a vaccine to be used in humans, is no longer necessary and may not be wise for two reasons. One is that the human cells involved in tuberculoimmunity can be cultured and studied in vitro, and most questions about such a vaccine can be studied in well-controlled, rapid tissue culture experiments (17, 18, 23, 25, 59, 60). The other is that long-suspected differences in mechanisms of pathogenesis and immunity between animals and humans have now been demonstrated at the molecular level. Thus, gamma interferon protects mouse but not human macrophages against tubercle bacilli, vitamin D metabolites enhance human but not mouse macrophage resistance to the bacilli, and glucocorticoids increase mouse but not human macrophage permissiveness in relation to the bacilli (61, 62; see also references 26, 28, and 59). This molecular evidence supports long-standing experience that results from animal or animal cell experiments cannot be relied on to produce information useful for immunization of humans and may actually be misleading.

In the likely event that the purified polysaccharide is unable to elicit suitable induction or response in human skin or in human cell cultures, research on binding this haptenic antigen to an appropriate adjuvant carrier will be necessary. The carrier could be chosen not only to enable proper presentation of the hapten for reaction but also for selective induction of a cell-mediated immunologic response. For example, the carrier could be a cationized protein. Such proteins have been known for some time to selectively and powerfully induce cell-mediated immune responses (20). They probably promote association of antigens with the membrane of antigen-presenting cells in the conformation specially needed for such induction (3).

Field testing an antituberculosis vaccine is prohibitively slow and expensive, and the results are frequently difficult to control and interpret. The new vaccine(s) may not have to be used in the field until there is considerable certainty about its actual effectiveness and knowledge of protocols for its best use in various sets of humans. Small numbers of subjects could be experimentally immunized, and development of immunity could be measured and monitored by in vitro testing (18, 20, 59). This kind of testing would allow adjusting the use of the vaccine for groups of subjects with diverse responsiveness to antituberculosis immunization, such as caused the selective failure of BCG in World Health Organization trials in India. Preliminary, rapid, well-controlled testing of this kind should remove what probably has been the largest barrier to improving antituberculosis immunization in humans—the active avoidance of testing in human subjects of any vaccines besides BCG because of the great cost and time required for field trials.

ACKNOWLEDGMENTS

Recent work in this laboratory relating to the subject of this review has been made possible by support from the John A. Hartford Foundation, the Robert J. Kleberg, Jr., and Helen C. Kleberg Foundation, the William H. Donner Foundation, the Denver Department of Health and Hospitals, and the IMMTUB program of the World Health Organization.

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