Current Status of Immunologic Approaches to Treating Tobacco Dependence: Vaccines and Nicotine-specific Antibodies

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ABSTRACT

In contrast to current pharmacotherapies, immunologic approaches to treating tobacco dependence target the drug itself rather than the brain. This approach involves the use of nicotine-specific antibodies that bind nicotine in serum, resulting in a decrease in nicotine distribution to the brain and an increase in nicotine's elimination half-life. This review summarizes the literature examining the effects of immunologic interventions on the pharmacokinetics and behavioral effects of nicotine in animal models, as well as recent phase I and II clinical trials in humans. Studies using various vaccines and nicotine-specific antibodies in rodents have shown that immunization can significantly reduce the behavioral effects of nicotine that are relevant to tobacco dependence (eg, nicotine self-administration). These findings provide proof of principle that immunologic interventions could have utility in the treatment of tobacco dependence. Thus far, phase I clinical trials of nicotine vaccines have not produced any serious adverse events in humans and have produced dose-dependent increases in serum antibody levels. Although preliminary data from these small trials suggest that vaccination can facilitate abstinence from tobacco use, more advance trials are needed. By acting outside the nervous system, immunologic approaches are less likely to produce the adverse side effects associated with current medications. In addition, the unique mechanism of action of immunotherapy makes it particularly suitable for combination with other pharmacological approaches. Taken together, the work completed to date provides substantial evidence that immunologic interventions could play an important role in future treatment strategies for tobacco dependence.

KEYWORDS: tobacco, nicotine, vaccination, passive immunization, antibodies, pharmacokinetics

INTRODUCTION

Tobacco dependence is the most common form of drug abuse. There are an estimated 71.5 million adult cigarette smokers, and smoking is associated with over 400 000 deaths per year in the United States.¹ Thus, treatment of tobacco dependence continues to be a major public health priority.² Nicotine is considered the primary chemical in tobacco that is responsible for engendering tobacco use and dependence.^{3,4} Although it is clear that other compounds in tobacco and nonpharmacologic variables can play a role in the development and maintenance of tobacco dependence, the primary role of nicotine has broad empirical support providing a strong rationale for targeting its effects in the development of interventions for tobacco dependence. Nicotine produces its addictive effects by altering neuropharmacological processes in the brain. For example, nicotine produces an increase in extracellular dopamine in the nucleus accumbens,⁵ an effect common with other drugs of abuse (eg, heroin, cocaine^{6,7}). Currently available pharmacotherapies for tobacco dependence involve administration of a medication that substitutes for or modifies some aspect of these neuropharmacological effects of nicotine. For example, the most commonly used medication for smoking cessation is nicotine replacement therapy (NRT), which involves delivery of pure nicotine via transdermal patches or other routes as a substitute for the nicotine otherwise derived from using tobacco. The atypical antidepressant bupropion, which primarily inhibits dopamine and norepinephrine transporters, is another first-line medication used for tobacco dependence.⁸ Both NRT and bupropion significantly increase quit rates over placebo.⁹⁻¹¹ However, despite their efficacy in promoting cessation, the vast majority of smokers that use NRT or bupropion fail to quit.^{8,10} There is a clear need for improved pharmacotherapies for nicotine dependence.

The approach of targeting the neuropharmacological actions of nicotine in the brain poses 2 significant challenges. First, the neuropharmacological mechanisms involved in nicotine's effects are also important in mediating normal functioning of the nervous system. Thus, medications targeting nicotine's neuropharmacological effects may alter normal functioning and produce undesired side effects. Second,

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nicotine acts through multiple neuropharmacological mechanisms to produce its addictive effects. Finding one receptor-based medication or a combination of such medications that targets more than one of these critical mechanisms is difficult. Development of alternative strategies that circumvent these challenges is of interest. One alternative is an immunologic approach, which targets nicotine itself rather than the brain. This approach involves the production of nicotine-specific antibodies in serum that bind nicotine in blood and reduce nicotine distribution to brain. As a result, vaccination can reduce the addictive effects of nicotine, and thereby facilitate treatment of tobacco dependence. By acting outside the brain, immunologic approaches should lack the central nervous system (CNS) side effects associated with other types of medications. By preventing nicotine distribution to brain, all of nicotine's neuropharmacological effects in brain are attenuated.

An immunologic approach to treating drug dependence was first suggested over 30 years ago, when it was reported that immunization against heroin could reduce the likelihood that monkeys would self-administer the drug. Since then, vaccines and drug-specific antibodies have been developed that are effective in modifying the pharmacokinetics and behavioral effects of a range of drugs of abuse in animals, including cocaine, phencyclidine, and methamphetamine.^{12,13} The purpose of the present article is to review the literature examining immunologic approaches against nicotine. Focus will be primarily on studies employing pre-clinical animal models, but brief coverage of recent early-phase clinical trials in humans will also be provided. Finally, several issues regarding the clinical application of immunologic interventions will be discussed.

MECHANISM OF ACTION

Nicotine, or any other drug of abuse, serves as a positive reinforcer. That is, it increases the frequency of behaviors, such as smoking tobacco, that lead to its delivery to the brain. This positive reinforcing effect of nicotine then leads to high sustained rates of smoking that are a hallmark of tobacco dependence and associated with many adverse health consequences (eg, lung cancer, cardiovascular disease). Thus, the positive reinforcing effect of nicotine on behavior is a cornerstone of tobacco dependence, and a primary behavioral target for medication development. Medications that attenuate nicotine's reinforcing effects should be useful in facilitating cessation of tobacco use.

The pharmacokinetic properties of drugs (ie, absorption, distribution, metabolism, elimination) are key determinants of their reinforcing effects. The dose of nicotine reaching the brain determines whether it has no effect, reinforcing effects, or punishing effects on behavior.^{5,14} The speed with which nicotine is absorbed and reaches the brain also deter-

mines the strength of its reinforcing effects. Smoking, which produces peak blood levels within 10 to 20 seconds, is more reinforcing than chewing nicotine gum, which produces peak blood levels within 15 to 30 minutes. Conversely, slower metabolism and elimination of nicotine has been associated with lower rates of smoking needed to maintain a desired blood level and rate of reinforcement.¹⁵⁻¹⁷ Because of their key role in determining the reinforcing effects of nicotine, these pharmacokinetic processes represent a potential pharmacological target for developing medications that attenuate nicotine's reinforcing effects and thereby facilitate smoking cessation. This is the primary goal of an immunologic approach to treating tobacco dependence.

Immunization against nicotine involves using nicotine-specific antibodies that bind nicotine in serum. In an immunized subject, nicotine-specific antibody is present in the bloodstream and extracellular fluid. The antibody is excluded from the brain because it is too large to cross the blood-brain barrier. When an immunized subject receives nicotine, a substantial fraction of the drug is bound to antibody, sequestered in blood, and prevented from entering the brain to thereby produce its reinforcing effects on tobacco use. In addition, the binding of nicotine by antibody makes it less available for metabolism. Thus, vaccination markedly slows nicotine's elimination half-life. Consequently, the rate of smoking could be reduced by prolonging the effect of nicotine from each cigarette and delaying the nicotine deprivation that leads to smoking the next cigarette. It is important to note that, although immunization against nicotine reduces the ability of the drug to bind to nicotinic cholinergic receptors, it should not be considered analogous to a nicotinic acetylcholinergic receptor antagonist such as mecamylamine. Receptor antagonists block the binding of endogenous compounds (eg, acetylcholine) to receptors, while antibodies do not. Moreover, nicotine-specific antibodies have the additional effect of increasing the elimination half-life of nicotine, while receptor antagonists do not.

IMMUNOLOGICAL METHODS

Active Versus Passive Immunization

Immunization against nicotine can be achieved by 2 methods. Active immunization (hereafter referred to as vaccination) involves repeated administration of an immunogen to the subjects being studied in order to stimulate the immune system to produce nicotine-specific antibodies. Passive immunization involves the production of antibodies in some other species (eg, rabbits) or in vitro, which are then purified and administered to the subjects being studied. Each method has advantages and disadvantages. Vaccination requires relatively few administrations (eg, 1 injection per month for 3 to 4 months) to produce a high serum level of antibody that persists for several months. It is also relatively

inexpensive. The primary disadvantages of vaccination are the delay to achieving required antibody levels and the inability to control those levels. Passive immunization also offers several advantages, including the ability to (a) achieve the required serum level of antibody virtually immediately, compared with the 1 to 2 months needed for vaccination, (b) control the antibody dose to study dose-response relationships, and (c) examine the effects of high antibody doses that cannot be achieved with vaccination alone. The primary disadvantages of passive immunization are that it requires more frequent injections to maintain required antibody levels and is more expensive than vaccination.

Vaccine Formulation and Administration

Nicotine is too small (molecular weight [MW] 167 kD) to elicit an immune response (ie, it is not immunogenic). Thus, regular tobacco users do not have antibodies against it. Nicotine is rendered immunogenic by conjugating (ie, linking) the drug itself or a structurally related compound (ie, hapten) to an immunogenic carrier protein to form a complete immunogen, referred to as a conjugate vaccine. Various types of carrier proteins have been employed, including keyhole limpet hemocyanin (KLH),¹⁸⁻²⁰ a 19-residue peptide,²¹ recombinant cholera toxin B subunit,²² and recombinant pseudomonas exoprotein A.²³ The latter 2 have the advantage of being used previously in vaccines administered to humans. The conjugation of nicotine to a carrier protein has typically been accomplished using a linker, such as succinic acid. One vaccine in development uses virus-like particles formed from the bacteriophage Qb instead of a carrier protein.²⁴ Most vaccines are prepared for administration by mixing the complete immunogen with an adjuvant (eg, Freund's in animals, alum in humans), which enhances the immune response. The peptide-based vaccine mentioned above does not use additional adjuvant.

After the initial injection of vaccine, periodic booster doses are needed to maintain satisfactory antibody levels, since exposure to nicotine by itself does not elicit an anamnestic (ie, booster response). Vaccination schedules in rats typically involve 2 to 4 injections at 2 to 4 week intervals. No studies have been published directly comparing different schedules to suggest an optimal one. Vaccination schedules during early clinical trials in humans have involved 2 to 6 injections also at 2 to 4 week intervals.

Specific Vaccines and Antibodies

The effects of 9 different nicotine vaccines have been reported in rodents,^{18-22,24-27} 3 of which have been tested in phase I and II clinical trials.^{24,28,29} The effects of passive immunization using various forms of nicotine-specific

antibody have also been examined in several studies in rodents.^{20,23,30,31} Although the formulation varies between these vaccines and antibodies, their mechanism of action is the same and their pharmacokinetic and behavioral effects in animals and humans are generally similar.

Antibody Characteristics

Three characteristics of vaccines that are relevant to treating drug abuse include its immunogenicity, and the affinity and specificity of the elicited antibodies. Immunogenicity refers to the serum concentration of antibody that is achieved. In order to be maximally effective, a vaccine must elicit and maintain a high serum concentration of antibody throughout the period of interest, because higher ratios of antibody to nicotine result in greater binding of nicotine in serum. Affinity refers to the strength with which the elicited antibodies bind the drug. Specificity refers to the extent to which the antibodies bind nicotine in preference to other compounds. Greater specificity reduces competition from other compounds for binding capacity, improves safety, and reduces the likelihood of adverse side effects. Vaccine formulation can influence these 3 properties. For example, specificity is influenced by linker position. Linkers that are distant from prime sites of metabolism help to elicit antibodies that preferentially bind nicotine over its metabolites.^{18,23} In addition, immunogenicity appears to be influenced by the design of the hapten.²⁶

All of the vaccines studied to date in animals have been sufficiently immunogenic to elicit significant concentrations of nicotine-specific antibody in serum (eg, 0.1-0.29 mg/mL^{18,32,33}) that bind nicotine with high affinity (eg, K_d 37-50 nM^{23,24}). These antibodies also generally show high specificity for nicotine, as binding of other compounds is very low (cross-reactivity with other compounds such as nicotine metabolites, acetylcholine, or other neurotransmitters is typically less than 5%^{18,19,22,24}).

PRECLINICAL STUDIES IN NONHUMANS

Pharmacokinetic Studies

The effects of vaccination or passive immunization on nicotine pharmacokinetics have been studied using both acute and chronic nicotine dosing protocols. Acute protocols have typically involved rapid delivery of a single intravenous (IV) dose of 0.01 to 0.03 mg/kg nicotine (equivalent on a weight basis to the nicotine absorbed from two thirds to 2 cigarettes by a smoker), with serum and brain nicotine levels measured 1 to 60 minutes postinfusion. Chronic protocols have involved either repeated bolus doses (0.003-0.03 mg/kg IV) or continuous infusion of nicotine via subcutaneous (sc) osmotic mini-pump (eg, 1.0 mg/kg/d). These protocols have been designed to achieve serum nicotine

concentrations approximating that of moderate to heavy smokers (10-40 ng/mL).

Nicotine binding in serum

The binding of nicotine to serum proteins is normally quite low (<10%). However, in rats immunized against nicotine, 80% to 99% of nicotine in serum is bound to nicotine-specific antibody.^{18,23,30} Consequently, studies have shown that total serum nicotine concentrations in immunized subjects are 300% to 850% higher than that in controls (Figure 1).^{22,23,32} This increase in nicotine binding results in up to a 92% decrease in the concentration of nicotine that is unbound in serum and capable of entering the brain.³³ In passively immunized rats, this effect has been shown to be directly related to antibody dose and affinity.³⁰

Nicotine distribution to brain

The significant decrease in unbound serum nicotine concentrations in immunized subjects results in an associated decrease in nicotine distribution to brain. Across various animal studies, brain nicotine concentrations are 40% to 92% lower in vaccinated subjects compared with controls within the first few minutes after a single nicotine dose.^{22,32} Similar results have been obtained with passive immunization (30%-90% reduction,^{20,23,30} Figure 1). This attenuation of the early distribution of nicotine to brain is particularly important because the positive subjective and reinforcing effects of nicotine are apparent within minutes after the first few puffs of a cigarette.³⁴ Several variables have been shown to influence the efficacy of immunization in reducing nicotine distribution to brain. First, effects are greatest in subjects with the highest serum concentration of antibody

and, thus, the greatest capacity to bind nicotine.³⁵ Second, when higher single nicotine doses are administered, the degree of reduction in nicotine distribution to brain can be somewhat less (58%).²⁰ Third, when nicotine is administered chronically via repeated bolus doses or a continuous infusion, immunization has relatively little effect on the chronic accumulation of nicotine in brain (23%-29% reduction^{35,36}). However, immunization still substantially reduces the peak level of nicotine produced by each individual dose. Finally, the efficacy in reducing nicotine distribution to brain has been shown to be directly related to antibody dose and affinity in passively immunized rats.^{23,30}

One clinical concern is that a smoker could overcome the reduced distribution of nicotine to brain by increasing nicotine intake (ie, compensating) and overwhelming the binding capacity of the antibody. As a result, the desired CNS effects of nicotine could still be achieved. However, studies in animals have shown that both the cumulative distribution of nicotine to brain and distribution of a single dose are significantly decreased even when the nicotine dosing protocol provides a total nicotine dose exceeding the estimated binding capacity of antibody by up to 33-fold.³⁶ This finding suggests that the immediate rewarding and reinforcing effects of nicotine could still be suppressed in the context of increased nicotine exposure (eg, compensatory smoking).

Nicotine elimination

Because bound nicotine is protected from metabolism and excretion, vaccination restricts metabolism to the unbound fraction of nicotine, thereby slowing the overall elimination of nicotine from the body. Studies have shown that nicotine clearance is reduced by 90%, and serum half-life is prolonged from 0.8 hours up to 6 hours after a single nicotine dose in immunized rats.^{35,37} As discussed above, this finding suggests that immunization could reduce smoking by prolonging the effects of nicotine and reducing the rate of nicotine intake required to obtain desired effects.

Behavioral Studies

The studies discussed above clearly demonstrate that immunization against nicotine can have profound effects on nicotine pharmacokinetics by reducing nicotine distribution to brain and slowing nicotine elimination. However, it is also clear that nicotine distribution to brain is not completely prevented, especially under some conditions of chronic nicotine exposure. Therefore, the question of whether these pharmacokinetic effects are large enough to alter the dependence-related behavioral effects of nicotine must be addressed. Significant progress has been made over the past 5 years addressing this question, as the effects of nicotine-specific antibodies have been characterized in a wide range

Figure 1. Mean ($\pm$ SEM) serum and brain nicotine concentrations in vaccinated and control rats. Three minutes after a single IV nicotine dose (0.03 mg/kg), the serum nicotine concentration was higher in vaccinated rats, and brain nicotine concentration was 64% lower than control rats. ***Significantly different from control, $P < .001$ (from Pentel et al, 2000²³ with permission from Elsevier).

of animal behavioral models. Some of these models (eg, locomotor behavior, seizures), although not particularly germane to the behavioral features of tobacco dependence, are useful for demonstrating that nicotine-specific antibodies can attenuate the behavioral effects of nicotine. The other models that have been used (eg, nicotine withdrawal, self-administration) are more relevant to tobacco dependence in that they model more closely the type of nicotine exposure and specific diagnostic criteria associated with tobacco dependence.

Locomotor behavior

As with other psychomotor stimulant drugs (eg, cocaine), nicotine induces an increase in locomotor activity when administered to rodents in a familiar environment.³⁸ To demonstrate this effect, rats are initially habituated to (ie, familiarized with) an activity test chamber for 3 sessions. Nicotine or saline is then administered prior to a test session and an activity score is obtained. Rats exposed to nicotine exhibit significantly higher levels of locomotor activity compared with saline-treated rats (Figure 2). Passive immunization against nicotine has been shown to block this locomotor-stimulant effect of a single dose of nicotine, while cocaine-induced locomotor activity is unaffected.²³ Thus, the effects of passive immunization are specific to nicotine's locomotor stimulant effects as opposed to drug-induced locomotor stimulation in general.

With repeated administration of the same dose, the locomotor-stimulating effect of nicotine progressively increases (ie, locomotor sensitization is observed).³⁸ The putative neural substrate that mediates this effect is the mesolimbic

dopamine system, the same system involved in the reinforcing effects of nicotine. The neuroadaptations that result from repeated exposure to nicotine may render this system more sensitive to the drug. As such, immunization may be less effective in attenuating the locomotor stimulant effects of nicotine in sensitized rats. However, both vaccination and passive immunization have been shown to attenuate the locomotor-stimulant effects of nicotine in rats after repeated exposure to nicotine, with passive immunization doing so in dose-dependent fashion.²⁰

Nicotine-induced seizures

At toxic doses, nicotine can induce seizures in both humans and animals. The large doses required to induce seizures in rats provide a rigorous test of an antibody's ability to reduce nicotine distribution to brain and nicotine-induced behavioral effects. In one study, vaccination was shown to reduce the incidence of seizures and distribution of nicotine to brain following a dose (2 mg/kg) that was 28-fold higher than the estimated binding capacity of the antibody.³⁹ The serum nicotine concentration produced by this dose in control rats (1050 ng/mL) was 25 to 50 times that of a regular smoker (10 to 40 mg/kg). This finding correlates well with pharmacokinetic studies showing continued efficacy despite a high degree of saturation of antibody and demonstrates that vaccination can effectively reduce the behavioral effects of nicotine even when such large nicotine doses are administered.

Nicotine relief of nicotine withdrawal syndrome

Part of the diagnostic criteria for tobacco dependence is the presence of a withdrawal syndrome upon termination of tobacco use. This withdrawal syndrome is characterized by several somatic and affective signs and symptoms, including gastrointestinal discomfort, depressed mood, irritability, anxiety, difficulty concentrating, and craving for tobacco. Tobacco withdrawal is important because many studies have shown it is one factor that can motivate relapse and undermine cessation success.⁴⁰ For example, smokers who experience withdrawal upon quitting may relapse because the nicotine obtained from smoking terminates the aversive withdrawal symptoms. This relief of withdrawal serves as another source of reinforcement that can maintain tobacco use. Consistent with this notion is that nicotine replacement products promote cessation in part because they relieve withdrawal symptoms. The ability of nicotine to relieve withdrawal can be modeled in animals. Rats are first made dependent on nicotine by administering a continuous sc nicotine infusion for 1 week. Termination of the infusion then results in a withdrawal syndrome characterized by a range of behavioral signs (eg, writhing, body shakes, tremors). If nicotine is administered again, the frequency of

Figure 2. Mean ($\pm$ SEM) change in locomotor activity in rats after nicotine administration (0.28 mg, sc). In rats that were not vaccinated (open bars), nicotine-induced a significant increase in locomotor activity. However, nicotine-specific antibody (Nic-IgG) completely blocked the nicotine-induced increase in activity observed in control rats. *Significantly different from control IgG + nicotine, $P < .05$ (from Pentel et al, 2000²³ with permission from Elsevier).

behavioral signs of withdrawal significantly decreases. However, in passively immunized rats, nicotine fails to reduce signs of withdrawal (Figure 3).³¹ Thus, to the extent that withdrawal motivates tobacco use, this finding suggests that immunization against nicotine could reduce the likelihood of relapse by rendering tobacco use less effective in relieving withdrawal.

Subjective effects

When humans use tobacco, they report various sensations or subjective effects. To the extent that these effects play a role in facilitating the development and maintenance of tobacco dependence, blocking these effects may promote cessation of tobacco use. Animals can be taught to distinguish between the subjective effects of nicotine and other compounds using a drug discrimination procedure. This technique involves training rats to press one lever (nicotine lever) in a test chamber after administration of a dose of nicotine, and a different lever (saline lever) after administration of saline. In well-trained rats, nicotine comes to serve as a discriminative stimulus, such that the majority of lever presses will occur on the nicotine lever when nicotine is administered. Moreover, when the dose of nicotine is decreased, lever pressing decreases on the nicotine lever and increases on the saline lever, demonstrating that the subjective effects of nicotine are dose related. In one study using this paradigm, rats that were passively immunized against nicotine emitted fewer responses on the nicotine lever after a nicotine injection than control rats (Figure 4).⁴¹

Figure 3. Mean ($\pm$ SEM) overall abstinence signs following sc injection of either nicotine (0.12 mg/kg) or saline during nicotine withdrawal in rats. Values are expressed as a percentage of signs observed prior to injection. Rats were passively immunized 25 hours earlier with either nicotine-specific antibody (Nicotine IgG) or control immunogen (Control IgG). Nic-IgG completely reversed the decrease in abstinence signs produced by nicotine that was observed in rats administered control IgG.

*Significantly different from control IgG + nicotine, $P < .05$ (from Malin et al, 2001³¹ with permission from Elsevier).

Figure 4. Mean ($\pm$ SEM) percentage of total responses made on the nicotine lever as a function of nicotine dose in immunized and control rats. Immunized rats exhibited a significantly lower percentage of responses on the nicotine lever following nicotine administration than control rats. *Significantly different from control, $P < .05$ (from Malin et al, 2002⁴¹ with permission from Elsevier).

Another study using a related paradigm showed that the rate of acquisition of a nicotine discrimination was slower in vaccinated rats.²¹ Thus, both vaccination and passive immunization can attenuate the subjective effects of nicotine. This finding suggests that immunization against nicotine might reduce the pleasurable subjective effects of tobacco use and thereby facilitate cessation.

Reinforcing Effects

As discussed above, a hallmark of drug dependence is the high rate with which the drug is self-administered. Most drugs that are abused by humans, including nicotine, are also self-administered by animals. As such, nicotine self-administration in animals is considered a preclinical model of tobacco dependence, and studies of such behavior are thought to be relevant to the analysis and treatment of this disorder.⁵ In a nicotine self-administration (NSA) procedure, IV nicotine infusions are administered contingent upon the animal emitting a specified response (eg, lever press). Both rodents and primates will exhibit robust levels of responding for nicotine infusions, and medications that reduce NSA in animals are thought to have potential utility in facilitating cessation of tobacco use in humans. Three phases of NSA often examined are acquisition, maintenance, and reinstatement. These 3 phases are considered relevant to similar phases of tobacco use in humans, such as initiation of use, continuation of use, and relapse after a quit attempt, respectively. The effects of nicotine vaccines on each of these phases of NSA have been examined.

In a recent study examining acquisition of NSA,²⁵ rats were vaccinated with nicotine or control immunogen prior to being given access to nicotine for 3 weeks. NSA was significantly lower in vaccinated rats compared with controls during the acquisition period, with vaccinated rats earning 38% fewer infusions during the last week of training (Figure 5A)

Figure 5. Effects of vaccination on nicotine and cocaine self-administration. One week after their fourth and final vaccine dose, rats were placed in operant chambers where they had access to IV drug infusions 23 h/d. For the first week each response on an active lever (FR 1) produced an infusion of drug (0.01 mg/kg nicotine or 0.2 mg/kg cocaine). In the second and third weeks, 2 (FR 2) or 3 (FR 3) responses respectively were required for each infusion. (A) Number of nicotine infusions earned in immunized and control rats during the acquisition period. The dotted line indicates the minimum number of infusions required to meet acquisition criteria. At the end of week 3, responding was significantly lower in immunized rats compared with controls ($*P < .05$). (B) Data for 5 vaccinated rats, which did meet acquisition criteria are shown. Their rates of NSA were similar to those of controls, suggesting that compensation did not occur. (C) Number of cocaine infusions in immunized and control rats during acquisition. No significant effect of vaccination on acquisition of cocaine self-administration was observed (from LeSage et al, in press, with permission from Springer Science and Business Media).

The percentage of rats meeting acquisition criteria in the vaccinated group was lower (36%) than that in the control group (70%), but this difference was not statistically significant. In the subgroup of rats that acquired NSA, infusion rates were similar to those in control rats (Figure 5B), indicating that this subgroup did not compensate by increasing

their infusion rate to surmount the effects of vaccination on nicotine pharmacokinetics. In a parallel experiment, vaccination did not affect acquisition of cocaine self-administration, demonstrating its specificity for nicotine (Figure 5C). These findings suggest a potential role for vaccination in the prevention of tobacco dependence.

In the same study, the effects of vaccination on the maintenance of NSA were also examined. Rats were initially trained to self-administer nicotine, and then vaccinated with nicotine or control immunogen, while NSA continued to be monitored. The effects of vaccination on food-maintained responding were similarly examined to determine the specificity of vaccination effects. NSA was significantly reduced in vaccinated rats compared with controls after the final (4th) vaccine injection, with a mean reduction of 57% (Figure 6A). The slow onset of effect

Figure 6. Effects of vaccination on the maintenance of nicotine self-administration and responding for food pellets. Rats were initially trained to lever press for nicotine infusions (0.03 mg/kg/infusion) or food pellets. Responding was then extinguished and reacquired. After reacquisition of responding, rats received 4 doses of nicotine or control vaccine, while continuing to have access to nicotine (A) or food (B). Nicotine self-administration was reduced after the fourth vaccine dose in immunized rats compared with controls ($P < .05$). Immunization had no significant effect on food-maintained responding (unpublished data). *Significantly different from control, $P < .05$. (from LeSage et al, 2005, with permission from Springer Science and Business Media).²⁵

may have been owing to the slow development of antibody levels (and thus reduction of the nicotine dose reaching the brain) or that some nicotine still reaches the brain. Moreover, NSA has been shown to be less sensitive to changes in dose than self-administration of other drugs of abuse,⁵ and this may have also contributed to the slow onset of effect. No effect on food-maintained responding was observed (unpublished data, 2005, Figure 6B). There was no evidence that vaccinated rats attempted to compensate for altered nicotine distribution, as no significant increase in NSA was observed at any point during the vaccination period. These findings suggest a potential role for vaccination in facilitating cessation or reduction of tobacco use.

The effects of vaccination on reinstatement of NSA have also been examined. In this model, after rats are trained to self-administer nicotine, saline is substituted for nicotine to extinguish responding to low levels. Once extinction is achieved, a single priming dose of nicotine is administered prior to an extinction session. Reinstatement is evident when the priming infusion of nicotine produces a significant increase in responding on the lever that produced nicotine infusions during training. In one study,⁴² rats were vaccinated with nicotine or control immunogen during the extinction period prior to the reinstatement test. While control rats exhibited a significant increase in responding following a low priming dose of nicotine, vaccinated rats did not. Thus, vaccination blocked the ability of a low dose of nicotine to reinstate NSA. This finding suggests a role for vaccination in preventing relapse to tobacco use.

Although the findings from animal studies are encouraging and suggest a potential role for immunologic approaches to treating or preventing tobacco dependence, they should be interpreted with some caution. In some studies employing passive immunization, the antibody affinity and dose used was higher than that which has been achieved by vaccination, which could possibly exaggerate efficacy. However, the nicotine dose used in some of these studies was much higher than those in smokers, thus providing a rigorous test of immunization. Although some of the animal models discussed above are somewhat relevant to essential features of tobacco dependence (nicotine withdrawal, NSA), their relevance to tobacco use in humans is not entirely clear. These models differ from tobacco use in humans along several dimensions, including the route of nicotine administration, nicotine dose, and patterns of administration. In addition, the potential influence of non-pharmacological variables (sensory effects of smoke, social factors) that are known to promote tobacco use in humans on the efficacy of immunization remains to be determined. Development of better animal models may be helpful, but ultimately clinical trials in humans are needed to address some of these issues.

STUDIES IN HUMANS

Immugenicity and Adverse Effects

The results of phase I and II clinical trials have been reported for 3 nicotine vaccines: NicVAX,²⁸ NicQb,²⁴ and TA-NIC.²⁹ The vaccination schedule in these clinical trials consisted of 2 to 6 doses of vaccine at an interval of 2 to 4 weeks, and a later booster dose was administered in 2 trials.^{27,28} As in animal studies, serum antibody levels were low after the first dose and increased significantly after each subsequent dose. Marked variability in antibody levels between subjects has been observed. Antibody levels decreased by 50% over 6 to 8 weeks after the last vaccine injection of the initial immunization period but increased again when a booster dose was administered.²⁸ Thus, periodic booster doses would be needed to maintain antibody levels above some minimally acceptable value. In one trial, antibody levels in some subjects were in the range of that found to be effective in animal studies.²⁸ However, it is not yet clear what level of antibody will be necessary for efficacy in humans (see Clinical Issues).

No adverse events have been reported other than local reactions (eg, soreness or redness at the injection site) and mild systemic reactions (eg, flu-like symptoms such as fever, headache, malaise). Occurrence of these symptoms in placebo and vaccine groups was not significantly different, suggesting that they were the result of the adjuvant used rather than the immunogen. All symptoms resolved within 1 to 4 days without medical intervention. The lack of adverse effects is consistent with animal studies, demonstrating the specificity of antibodies for binding nicotine rather than endogenous compounds such as acetylcholine. These findings suggest that nicotine vaccines are well tolerated in humans and support the notion that immunotherapy may lack the side effects associated with other types of medication.

Potential Efficacy

Although these phase I and II clinical trials were not designed to examine the efficacy of vaccination in facilitating reduction or cessation of tobacco use, smoking status was nonetheless a secondary end point, thus providing a preliminary indication of efficacy.

In the TA-NIC trial,²⁷ participants were instructed to quit after receiving 6 vaccine injections at 1 of 3 doses over 12 weeks and were then boosted at 32 weeks. Quit rates were greater in the group receiving the highest vaccine dose than in the control group (38% vs 8%), but group size was small and statistical analysis was not reported. In the NicQb trial, participants who were motivated to quit were instructed to do so after the second monthly vaccine injection and received 3 additional monthly injections thereafter. Only a single

vaccine dose was tested. Smoking cessation counseling was provided to all participants at each visit during the first 3 months. Although abstinence rates confirmed by self-report and expired air CO did not differ between the vaccine and control groups, a subgroup of vaccinated participants with the highest antibody levels exhibited significantly higher abstinence rates than controls (57% vs 31%, $n = 30/53$ vs $25/80$). In the NicVax trial,²⁸ participants were neither motivated to quit nor instructed to do so. Participants received 3 monthly vaccine injections at 1 of 3 doses followed by a booster dose at 6 months. A greater percentage of subjects achieved 30 days of continuous abstinence (confirmed by self-report and expired air CO) in the high-dose vaccine group (40%) compared with the control group (8%). There was no evidence of increased smoking according to self-report, expired air CO, and urinary NNAL (a tobacco carcinogen nicotine metabolite). Thus, smokers did not try to compensate for possible reductions in nicotine distribution to brain.

It is important to stress that these initial clinical findings are preliminary, owing to small sample sizes and the necessity to stratify vaccinated participants based on antibody level to observe differences between groups. However, it is encouraging to see that despite the marked differences between these studies (ie, the motivation of participants to quit, whether they were instructed to do so, and whether they received concurrent counseling), they all showed a similar trend toward efficacy. Moreover, the fact that efficacy was only achieved in participants receiving the highest vaccine dose or achieving the highest antibody levels is consistent with animal studies showing that the greatest effect of vaccination in altering nicotine pharmacokinetics is observed in rats with the highest antibody levels.^{33,35} Thus, development of improved vaccine formulations or vaccination schedules that elicit higher antibody levels may be necessary to improve efficacy.

CLINICAL ISSUES

Advantages of Immunologic Approaches

As discussed above, immunologic approaches to treating tobacco dependence have 3 key advantages. First, immunization appears to be safe because of its low cross-reactivity with compounds other than nicotine. Second, immunization only requires a brief series of monthly injections to produce effects that can endure for months. The lack of major side effects and relatively minimal dosing requirements could be associated with improved patient compliance. Third, its unique mechanism of action makes it well suited for combination with other pharmacotherapies. Both the animal and human data thus far show that the efficacy of vaccination is limited. Despite best efforts to improve on immunologic methods in their own right, combining immunization with other medications may be necessary to maximize efficacy.

Potential Concerns

The lack of control over antibody levels and large variability between subjects is the primary limitation of vaccination. Both the animal and human studies discussed in this review show that achieving the highest antibody levels possible will be essential to maximizing the efficacy of vaccination. In addition, the slow development of antibody levels and onset of effect could discourage tobacco users who are eager to quit from trying vaccination, as treatment would need to be initiated months before the quit attempt. Methods of boosting immunogenicity need to be explored in order to address these issues. Passive immunization with a high-affinity antibody could be combined with vaccination to provide any desired antibody level and an immediate onset of effect. However, passive immunization is much more expensive and requires more frequent dosing, and potential side effects could occur (eg, allergic reactions).

To the extent that nicotine plays a role in the adverse effects of maternal smoking on fetal outcomes, immunization against nicotine could play a role in protecting the fetus from some of these adverse effects. Studies are needed to assess the safety of immunizing pregnant smokers and the efficacy of immunization in reducing fetal exposure to nicotine. Animal studies have shown that immunization reduces nicotine distribution to maternal brain in pregnant female rats to a similar extent as in male rats.^{37,43} In addition, immunization reduces nicotine distribution to fetal brain by up to 63% after a single nicotine dose. Although nicotine distribution to whole fetus is not reduced, immunization reduces the concentration of unbound nicotine in fetal serum. These findings suggest that immunization should not exacerbate the adverse effects of prenatal nicotine exposure and comment on the safety of immunizing pregnant smokers against nicotine.

There is concern that compensatory increases in smoking could occur to surmount the effects of immunization, possibly leading to increases in exposure to other harmful constituents in tobacco. However, there has been no evidence of compensation in either animals self-administering nicotine or smoking in humans. It is also possible that immunization could precipitate withdrawal. Although this has not been examined in animal models of nicotine withdrawal, one clinical trial found no evidence of vaccination precipitating withdrawal.²⁸

Vaccination would not address the effects of nicotine withdrawal, such as craving or negative affect, that would likely occur upon cessation of tobacco use. As discussed above, withdrawal can be a strong motivator for relapse and dropping out of treatment. Although immunization may attenuate the ability of a smoking lapse to relieve withdrawal, the withdrawal may be severe enough for a smoker to abandon continuing treatment. Thus, combining vaccination with

therapies directed against withdrawal processes may be helpful or even necessary for vaccination to be effective in treating tobacco dependence. Fortunately, this should be feasible owing to the unique mechanism of action of immunization and the low probability of adverse interactions that can be associated with combining other forms of pharmacotherapy.

Immunologic interventions target the role of nicotine in tobacco addiction. However, nonpharmacologic factors such as the environmental context and the sensory aspects of smoking also clearly play a role in tobacco dependence. These stimuli come to serve as powerful conditioned reinforcers that help to maintain tobacco use. Although immunization does not target these factors directly, it can help to alter the control of these stimuli. Whenever tobacco use occurs in a vaccinated user and nicotine cannot produce its reinforcing effects, then the association between nicotine at these nonpharmacological stimuli is broken and the stimuli should eventually lose their conditioned reinforcing strength.

Finally, other compounds in tobacco, such as acetaldehyde or nornicotine, may also play a role in the reinforcing effects of tobacco use. Although vaccination against nicotine will not impact these factors, it could be combined with other pharmacotherapies that do.

CONCLUSION

Immunization against nicotine can significantly attenuate several behavioral effects of nicotine in animals that are considered relevant to tobacco dependence in humans. These findings suggest that immunologic interventions could have use in the treatment of tobacco dependence. Initial clinical trials have demonstrated that nicotine vaccines are safe and produce substantial serum levels of nicotine-specific antibody in humans. Although preliminary data from these small trials suggest that vaccination may facilitate abstinence from tobacco use, more advance trials are needed to validate this finding. Taken together, the research to date suggests that immunological interventions could play an important role in future treatments for tobacco dependence. The primary role of such interventions will likely be in preventing relapse in smokers who are motivated to quit. By preventing a lapse from producing positive subjective and reinforcing effects, vaccination might prevent progression to full relapse. Another potential role for immunologic interventions is in facilitating reduction of tobacco use in people who are unwilling or unable to quit. It is generally accepted that the most effective approach to treating tobacco dependence is concurrent use of medications and behavioral therapy. Despite the significant therapeutic potential of immunological interventions, they do not target the nonpharmacological factors that maintain tobacco dependence and will likely be

maximally effective when combined with behavioral interventions that motivate abstinence from tobacco use.

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