

Metabolic, Humoral, and Cellular Responses in Adult Volunteers Immunized with the Genetically Inactivated Pertussis Toxin Mutant PT-9K/129G

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Summary

PT-9K/129G, a nontoxic mutant of pertussis toxin (PT) obtained by genetic manipulation, has been shown in animal models to be a promising candidate for new vaccines against whooping cough. To assess the safety and the immunogenicity of PT-9K/129G in humans, a pilot study has been performed in adult volunteers. The protein was found to be safe, capable of inducing high titers of toxin-neutralizing antibodies, and capable of generating immunological memory. In fact, vaccination caused an increase of cell-mediated response to PT, PT-9K/129G, S1 subunit, and B oligomer, indicating that memory T cells are induced by the vaccine. Since PT-9K/129G is mitogenic for T lymphocytes *in vitro*, it was investigated whether this activity is also present *in vivo*. No variation was observed in the proportion of T cells (CD3⁺), T helper cells (CD4⁺), and cytotoxic T cells (CD8⁺), as well as in that of other lymphoid populations, by FACS analysis. Interestingly, no thorough correlation was found between humoral and cellular responses. In one case, a very high cellular response was present in absence of detectable antibodies, suggesting that the antibody response, which is the only parameter measured in most clinical trials, may not give a complete picture of the response induced by a vaccine.

Although the conventional vaccine against whooping cough, composed of whole *Bordetella pertussis* bacterial cells, is very efficacious in preventing disease, it is not widely used, because immunization with this vaccine is associated with an excessive frequency of local and systemic side effects (1-3). As a result, pertussis is responsible for >60 million cases and more than half a million deaths each year (4). With the aim of developing safer and less reactogenic vaccines, many *B. pertussis* antigens have been purified and tested for safety and efficacy in animal models (5) and human clinical trials (6, 7). As anticipated by Pittman many years ago (8, 9), the results have shown that vaccines containing chemically detoxified pertussis toxin (PT),¹ either alone or combined with other antigens, are less reactogenic and can prevent children from having severe disease with an efficacy similar to that of the whole cell vaccine (7, 10). In spite of the efficacy shown in the clinical trial, acellular vaccines containing PT have not yet been licenced, because there are still doubts about their safety and their mechanism of protection. In fact, during a

large scale clinical trial carried out in Sweden, the chemically detoxified PT showed reversion to toxicity (11). Furthermore, no correlation was found between the level of antibodies against PT and protection from disease (7). To solve the problem of reversion to toxicity, we have developed an alternative method to detoxify PT. By means of genetic manipulation, we have modified two codons in the gene of PT and obtained a *B. pertussis* strain that secretes a PT molecule (PT-9K/129G) naturally devoid of toxicity (12). This molecule is an ideal candidate for a new vaccine against whooping cough since it does not require chemical treatment. Moreover, it cannot revert to toxicity and retains the natural B and T cell epitopes of PT, which may be lost during chemical detoxification. In addition, PT-9K/129G retains other properties of PT, such as the ability to bind the receptors on eukaryotic cells and to be mitogenic for T cells that are not present in chemically detoxified molecules. After extensive studies in animal models, which have shown that PT-9K/129G is nontoxic, immunogenic, and is able to protect mice from the infection with virulent *B. pertussis* (13), we have tested PT-9K/129G in human adult volunteers. Immunization with this antigen, which binds eukaryotic cell receptors and, at high doses, is mitogenic for T cells, raises questions never encoun-

¹ Abbreviations used in this paper: CHO, Chinese hamster ovary; FHA, filamentous hemagglutinin; PT, pertussis toxin; PTA, PBS containing 0.05% Tween 20 and 0.02% sodium azide.

tered before with conventional vaccines. To answer these questions, the clinical trial was designed not only to determine by conventional methods the safety and the immunogenicity of the candidate vaccine, but also to address the *in vivo* role of the T cell mitogenicity, and to find new parameters that might correlate with immunity against pertussis.

Materials and Methods

Subjects and Vaccination Schedule. 29 healthy adult volunteers of both sexes, 25–58 yr of age, recruited from Sclavo personnel, were randomly attributed to receive, in double-blind tests, PT-9K/129G or a placebo. All the volunteers had low anti-PT antibody titer (<20 EU/ml). 18 subjects received intramuscularly one dose of 0.5 ml of vaccine; 11 subjects received a placebo. After 6 wk, 17 subjects of the vaccine group and nine of the placebo group received, respectively, a second dose of vaccine or placebo with the same procedure used for the first injection. Two subjects of the placebo group and one of the vaccine group were not available when the second dose was administered, and therefore received only one dose. Informed consent was obtained from each volunteer. The protocol was approved by the New Drug Committee of the Italian Ministry of Health.

Vaccine. The acellular pertussis vaccine PT-9K/129G (lot no. D5/FA), prepared on June 22, 1989, and the placebo vaccine (lot no. 1/89S) were prepared at Sclavo Laboratories (Siena, Italy). Each 0.5-ml single-dose vial of vaccine contained 15 µg of PT-9K/129G, 0.05 mg of thimerosal, and 0.5 mg of aluminum hydroxide. Each 0.5-ml single-dose vial of placebo contained 0.05 mg of thimerosal and 0.5 mg of aluminum hydroxide.

Safety Assessment. Donors were monitored for adverse reactions and specific immune responses, as shown in Table 1. Blood samples for analysis of red and white blood cells, platelet count, hemoglobin level, hematocrit, glucose, urea, creatinine, SGOT, SGPT, γ GT, IgE, and insulin (fasting values) were obtained before each injection and after 3 d (also after 1 mo for IgE and insulin only). Body temperature was measured before each injection, after 6 and 24 h; injection sites were inspected daily for 3 d after each administration by a physician to monitor the presence of local reactions (erythema, induration, pain). A questionnaire was given to each volunteer on which to record reactions for a period of 2 wk.

FACS Analysis. Phenotypic analysis of whole blood cells was performed by direct immunofluorescence on a FACS (FACstar; Becton Dickinson & Co., Erembodegem, Belgium). The following PE- or fluorescein-conjugated mAbs were used: α-CD3 (OKT3); α-CD4 (OKT4) (Ortho Diagnostic Systems Inc., Raritan, NJ); α-CD8 (Leu-2a); α-CD25 (IL-2R); α-CD19 (Leu-12); α-CD23 (Leu-20); α-CD14 (Leu-M3); and α-CD57 (Leu-7) (Beckton Dickinson & Co.), as well as α-γ/δ receptor (TCR-δ1) (T Cell Sciences, Cambridge, MA) at appropriate dilution.

Antigens. Wild-type PT and the nontoxic mutant PT-9K129G were purified from the culture supernatants of the wild-type strain *B. pertussis* W28 and of the recombinant strain *B. pertussis* W28-9K/129G (12), by Affi-Gel blue absorption and fetuin-sepharose affinity chromatography (14). B oligomer was purified by the same method from the *B. pertussis* strain W28-8D/9G double mutant, which secretes into the culture medium only the B oligomer of PT (15). Subunit S1 of PT was expressed in *Escherichia coli* and purified as previously described (16). Filamentous hemagglutinin (FHA) was purified as described by Cowell et al. (17). All antigens used were >98% pure.

Chinese Hamster Ovary (CHO) Cell Toxin Neutralization Assay. Toxin-neutralizing antibodies induced by vaccination were tested by the CHO cell assay (18). Briefly, sera from volunteers obtained after vaccination with one or two doses of PT-9K/129G were diluted directly in the wells of flat-bottomed microplates (Costar, Cambridge, MA) in 25 µl of DME (Flow Laboratories, McLean, VA). Purified wild-type PT (120 pg) in 25 µl of DME (Coulter Immunology, Hialeah, FL) was added to each well, and the plates were incubated for 3 h at 37°C. After the incubation period, 0.2 ml of DME containing 10⁴ CHO cells, previously treated with 1 mg/ml of trypsin, was added to each well and incubated for 48 h at 37°C in atmosphere of 5% CO₂. As positive control, the clustering effect of PT alone was titrated in each plate. Neutralizing titers were expressed as the reciprocal of the highest serum dilution causing complete inhibition of the clustering activity induced by the native toxin. The U.S. Reference Pertussis Antiserum (human) containing 640 neutralizing units (NT), kindly provided by the Center for Drugs and Biologics (Bethesda, MD), was used as a standard.

ELISA. The ELISA method was performed as previously described for guinea pig sera (13). Wells of flat-bottomed polystyrene microtest plates (Dynatech Laboratories, Inc., Alexandria, VA) were

Table 1. Protocol for Clinical and Laboratory Assessment of the Response Induced by 9K/129G PT Vaccine

Response	Day										
	+1	+2	+3	+4	+30	+42	+43	+44	+45	+72	
Local reactions (pain, erythema, induration)	+	+	+	+		+	+	+	+		
Systemic reactions (>37.5°C)	+	+				+	+				
Leukocyte number	+			+	+	+				+	
Leukocyte markers	+			+	+	+				+	
Insulin, glucose*	+			+		+				+	
Antibodies	+				+					+	
Cellular immunity	+				+					+	

Injection I was given on day +1, and injection II was given on day +42.

* Fasting values.

coated with 100 μ l of PBS, pH 7.4, containing 1 μ g of purified PT or FHA. The coating was performed for 2 h at 37°C and overnight at 4°C in a humidified chamber. The coating buffer was aspirated, and wells were washed with 200 μ l of PBS containing 0.05% Tween 20 and 0.02% sodium azide (PTA). To minimize nonspecific adsorption of serum proteins to the plastic, wells were coated with 200 μ l of a blocking solution consisting of 1% BSA in PBS, and then incubated for 2 h at 37°C. Plates were then washed three times in PTA, and 200 μ l of fivefold diluted test serum were added to the wells. The U.S. Reference Pertussis Antiserum (human) containing 200 ELISA units per ml of IgG anti-PT and 200 EU/ml anti-FHA, kindly provided by the Center for Drugs and Biologics, was used as a standard. After incubation at 37°C, plates were washed three times with PTA, and a conjugate of anti-human IgG alkaline phosphatase was added. Plates were then incubated at 37°C and washed three times with PTA. Finally, 100 μ l of *p*-nitrophenyl phosphate substrate (Sigma Chemical Co., St. Louis, MO) (1 mg/ml in 1 M diethanolamine, pH 9.8) containing 1 mM MgCl₂, was added to each well. The enzyme-substrate reaction, which developed at room temperature, was stopped after 30 min, and the OD of the samples was measured at 405 nm against blank (substrate in diethanolamine, pH 9.8) on a Titertek Multiskan (Flow Laboratories, Inc.). Controls for each plate included wells with serum samples but no antigen, and wells with antigen but no serum samples. Each serum sample was tested in duplicate, and absorbance values were averaged. The calculation of the ELISA antibody units in the test samples was determined according to the U.S. Reference Pertussis Antiserum.

Proliferation Assays. Fycoll-Hypaque (Pharmacia Fine Chemicals AB, Uppsala, Sweden)-separated PBMC were incubated (10⁵ cells/well) for 3 d with PHA and 6 d with different antigen concentrations in flat-bottomed Cluster 96 plates (Costar). PT-9K/129G and B oligomer were heat inactivated (at 100°C for 45 min) to eliminate their mitogenic effect (19). The culture medium was RPMI 1640 (Gibco Laboratories, Paisley, Scotland) supplemented with L-glutamine (2 mM), 1% nonessential amino acids, 1% sodium pyruvate, 50 μ g/ml gentamycin, 5 \times 10⁻⁵ M 2-ME, and 10% pooled human AB serum. All assay cultures were pulsed for the last 16–18 h with 1 μ Ci of [³H]thymidine (sp act, 185 GBq/mmol; Amersham International, Amersham, UK). Cells were then harvested on glass fiber filters with a cell harvester (Skatron, Lier, Norway), and incorporated radioactivity was determined by liquid scintillation counting. Results of proliferation assays are presented as the mean counts per minute of triplicate cultures.

Statistical Analysis. The calculation of the ELISA antibody units was based on the parallel line bioassay procedure previously described using an ELISA unitage calculation program distributed by the Laboratory of Pertussis, Center for Drugs and Biologics (20). An increase in ELISA units of more than mean negative controls plus 3 SD was arbitrarily considered as a significant level of anti-PT and anti-FHA antibodies.

Results

Safety. A complete absence of typical adverse reactions, either local or systemic, was observed after the first dose of vaccine or placebo. After the second dose, 2 of 17 vaccinees had mild local pain, erythema, and induration, while only one of nine volunteers receiving the placebo had local pain. Hematological, clinical chemistry, insulin, and IgE values did not show any significant variation. No difference in leukocyte numbers or leukocyte population proportions was ob-

served between the two experimental groups (data not shown). Thus, these results in humans are similar to the animal studies, where the PT-9K/129G mutant was safe and unable to induce the leukocytosis that is typically observed with wild-type PT (12, 13).

Mitogenicity. Since it was observed that the mutant PT-9K/129G maintains the *in vitro* mitogenic activity of PT (13), a careful study was carried out with four vaccine and two placebo donors to clarify whether lymphoid subpopulation can be affected *in vivo* by the vaccine. Fig. 1 shows that either at 3 or 29 d after the first vaccine administration, the proportion of total T cells (CD3) or that of the Th (CD3/CD4) and T cytotoxic (CD3/CD8) subpopulation is almost identical. Similarly, the number of B cells (CD19), cells of the monocyte-macrophage series (CD14), and NK cells (CD57) remained the same after vaccination. Furthermore, neither activation markers, such as CD25 for T lymphocytes or CD23 for B lymphocytes, nor the proportion of T cells bearing the γ/δ receptor, changed after treatment (Fig. 1). It seems, therefore, that *in vivo* the mitogenic effect of PT-9K/129G does not play any relevant role.

Humoral Responses. Total antibodies against PT were evaluated by ELISA and CHO cell toxin neutralization assays (Fig. 2, A and B, respectively). In both cases, 17 of 18 vaccinees showed a statistically significant increase in titers after the first vaccine administration. The titers were not further boosted by the second injection. This was verified at an individual level (Fig. 2) as well as at a group level (Table 2). Total lack of correlation ($r = 0.277$, $p = 0.266$ for first injection; and $r = 0.191$, $p = 0.480$ for second injection) was observed between the two tests. Interestingly, a modest increase in antibody titer against FHA was observed by ELISA in vaccinees (Table 2). One of the vaccinees (V2) did not show any significant increase in antibody titer against PT (Fig. 2).

Cellular Responses. We have previously shown that donors that have acquired immunity against pertussis from the disease are able to express cellular responses to *B. pertussis* antigens (20), and that the cellular immunity against PT may be against the subunit S1 or B oligomer (19). In this study, we have investigated whether immunization with the genetically inactivated PT molecule elicits a similar cell-mediated response. Fig. 3 shows the results obtained with two representative donors. After vaccination, cell-mediated responses are increased against PT-9K/129G, wild-type PT, as well as against whole inactivated *B. pertussis*. Furthermore, the results suggest that immunity is directed against both the S1 subunit and the other subunits forming oligomer B. No modification of responsiveness to tetanus toxoid or PHA was observed. Almost all vaccinees tested did not possess cellular immunity against *B. pertussis* antigens at day +1 (Fig. 4 A) and converted to significant proliferative values at day +30 (Fig. 4 B). The magnitude of proliferative response to PT-9K/129G or wild PT can, however, differ from one donor to another (Fig. 4 B). In general, the highest proliferative responses against PT were observed in individuals with high antibody titers against this antigen. One exception was represented by donor V2. In fact, after repeated tests, this donor always showed

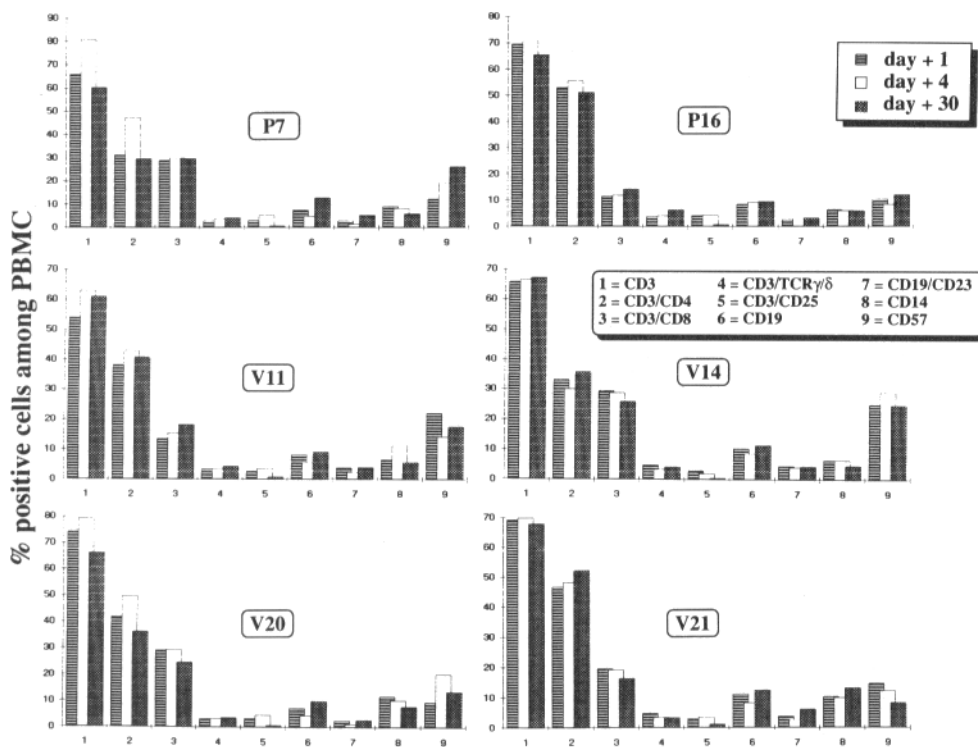


Figure 1. Phenotypic analysis of PBMC from four vaccinees (V11, V14, V20, and V21) and two placebos (P7 and P16). The analysis was performed before immunization (day +1), and 3 (day +4) and 29 (day +30) d after.

strong proliferative responses in the absence of specific antibodies against PT. Interestingly, this donor showed the same pattern of response, i.e., high proliferative response and very low antibody titer, even in response to tetanus toxoid (data not shown). On the other hand, donor V1 was able to produce high titers of antibody in spite of the very low proliferative response. It can be concluded that PT-9K/129G vaccine is in general able to induce both humoral and cellular responses, even though a direct correlation between the two cannot be observed.

Discussion

We have described the first experience of human immunization with a PT molecule that has been inactivated by genetic manipulation. Inoculation of humans with such a molecule has allowed us not only to ask whether it is safe, immunogenic, and therefore suitable for vaccine development, but also to ask other questions regarding the *in vivo* relevance of the mitogenic activity of PT and the immunogenicity of native molecules that, unlike the currently used vaccines, are not treated with chemical agents and still bind their receptors on eukaryotic cells. In addition to the parameters that are normally used to measure the immune response in vaccine clinical trials, we have measured the cellular immune response to the antigen, a parameter that is thought to be the *in vitro* correlate of the *in vivo* immunological memory. Interestingly enough, we have found that humoral and cellular responses do not necessarily correlate. This finding might be important to explain why no correlation has been found between

immune response and susceptibility to the disease in those vaccine trials where only antibody response has been tested (7).

Safety. PT-9K/129G vaccine did not induce significant adverse reactions and did not cause any change of the parameters tested, including the leukocyte, insulin, and IgE levels that are usually altered by PT (21). This results shows that the genetically detoxified PT-9K/129G can be safely used for human immunization and confirms the results previously obtained in animal models (13).

T Cell Mitogenicity. Several bacterial toxins produced by *Staphylococcus aureus* are potent T cell mitogens (22, 23). In some cases, their mitogenicity may be the cause of toxicity (24, 25). PT is a poor T cell mitogen and, to give a mitogenic effect *in vitro*, it requires doses that are $\sim 10^3$ – 10^6 times higher than those of staphylococcal enterotoxins SEA, SEB, or TSST-1. In fact, the mitogenic dose of TSST-1 is 1 pg/ml (25), 1 ng/ml for SEA and SEB (26), and 1 μ g/ml for PT (13). So far, the role in pathogenicity of the mitogenic effect of PT has never been investigated, because the toxicity of PT has always been attributed to the enzymatic activity of the S1 subunit. The availability of the PT-9K/129G mutant, devoid of enzymatic activity, but still mitogenic, has allowed us to investigate the *in vivo* role of the mitogenic properties of PT. We have previously shown that in mice PT-9K/129G does not cause any local or systemic effect (13). In this study, we have shown, through FACS analysis, that in humans the total number and subpopulation proportions of T, B, and NK cells, and monocytes remain unaltered after immunization with PT-9K/129G. Furthermore, activation markers, such as IL-2R (CD25) and lymphokine IgE receptor

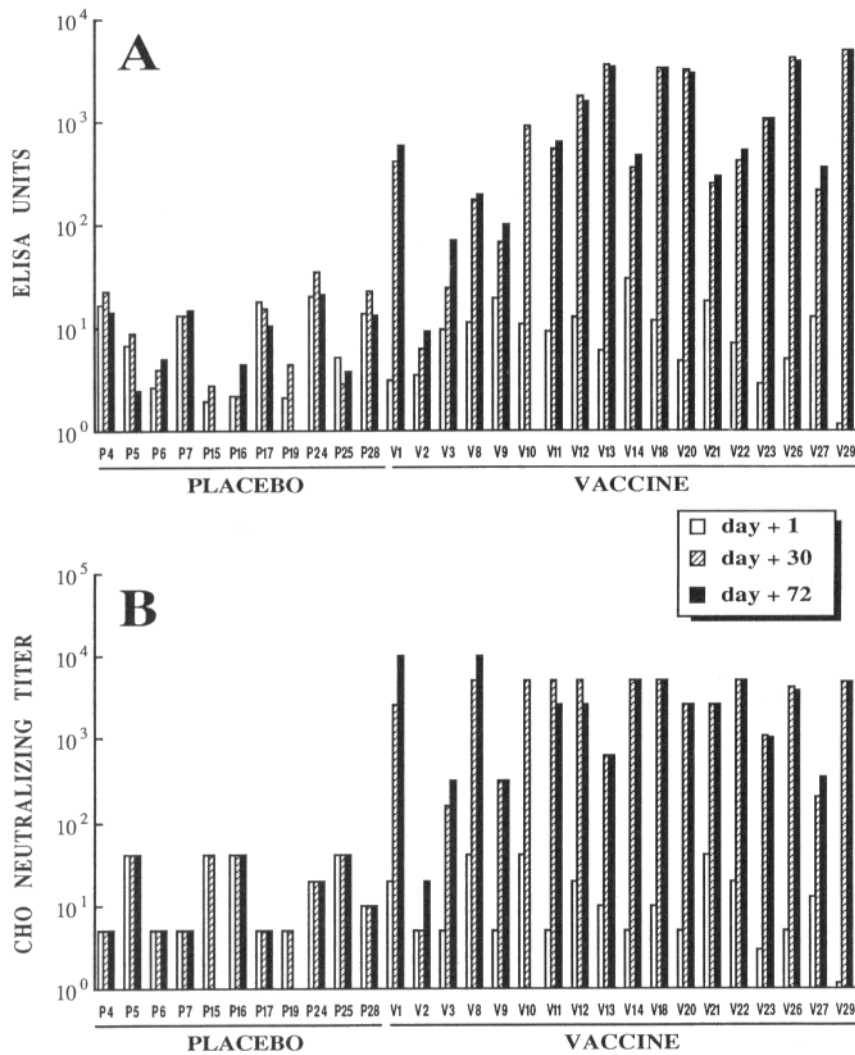


Figure 2. Antibody titers of volunteers measured by ELISA (A) and by CHO neutralizing test (B). Assays were performed before immunization (day +1), 29 d after the first immunization (day +30), and 28 d after the second immunization (day +72). Values are expressed in ELISA units or CHO neutralizing units. p, placebo; v, vaccine.

Table 2. Serum Antibody Responses in Adult Volunteers Receiving Placebo or PT-9K/129G Vaccine

Titer	Day	Placebo		Vaccine	
		Geometric mean (95% confidence interval)	Stimulation index (vs. day +1)	Geometric mean (95% confidence interval)	Stimulation index (vs. day +1)
Neutralizing titer (U in CHO assay)	+1	12.8 (7.5–21.6)		13.6 (8.6–21.5)	
	+30	12.8 (6.7–24.9)	1.0	1,810.2 (737.0–4,446.0)*	133
	+72	11.7 (5.9–23.0)	0.9	1,974.0 (820.1–4,751.4)*	145
Antibody titer anti-PT (Elisa U)	+1	6.5 (3.5–12.2)		6.7 (4.6–9.8)	
	+30	8.1 (4.2–15.5)	1.2	496.5 (199.9–1,233.3)*	74
	+72	7.9 (4.5–13.9)	1.2	522.4 (216.8–1,258.6)*	78
Antibody titer anti-FHA (Elisa U)	+1	9.4 (4.3–20.7)		12.7 (7.5–21.6)	
	+30	9.9 (4.7–20.9)	1.0	85.1 (54.9–131.7)*	6.7
	+72	8.4 (4.1–17.3)	0.9	57.7 (39.1–85.5)†	4.5

* $p < 0.01$ vs. day 0 value.

† $p < 0.05$ vs. day 0 value.

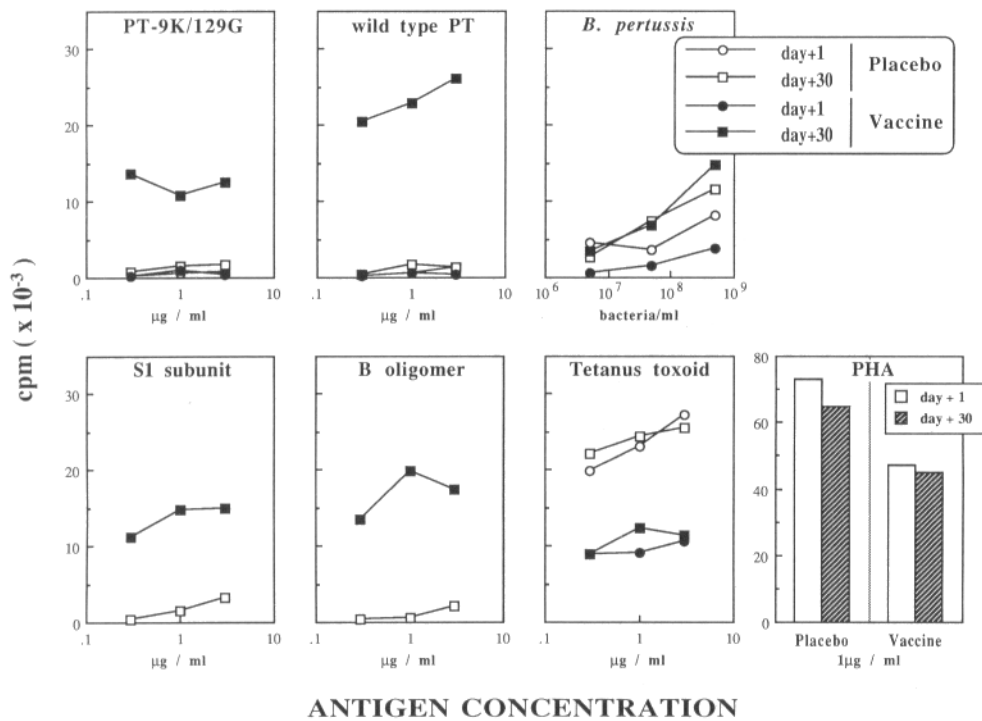


Figure 3. Proliferative responses of PBMC from one vaccinee and one placebo against PT, PT-9K/129G, *B. pertussis* cells, subunit S1, and B oligomer of PT. Tetanus toxoid and PHA were also tested as controls. The results shown for the two individuals (P16 and V11) are representative of those obtained with the other subjects (seven vaccinees and two placebos) that have been tested. Proliferation to the subunit S1 and to the B oligomer at day +1 were not done, since no response was observed with PT.

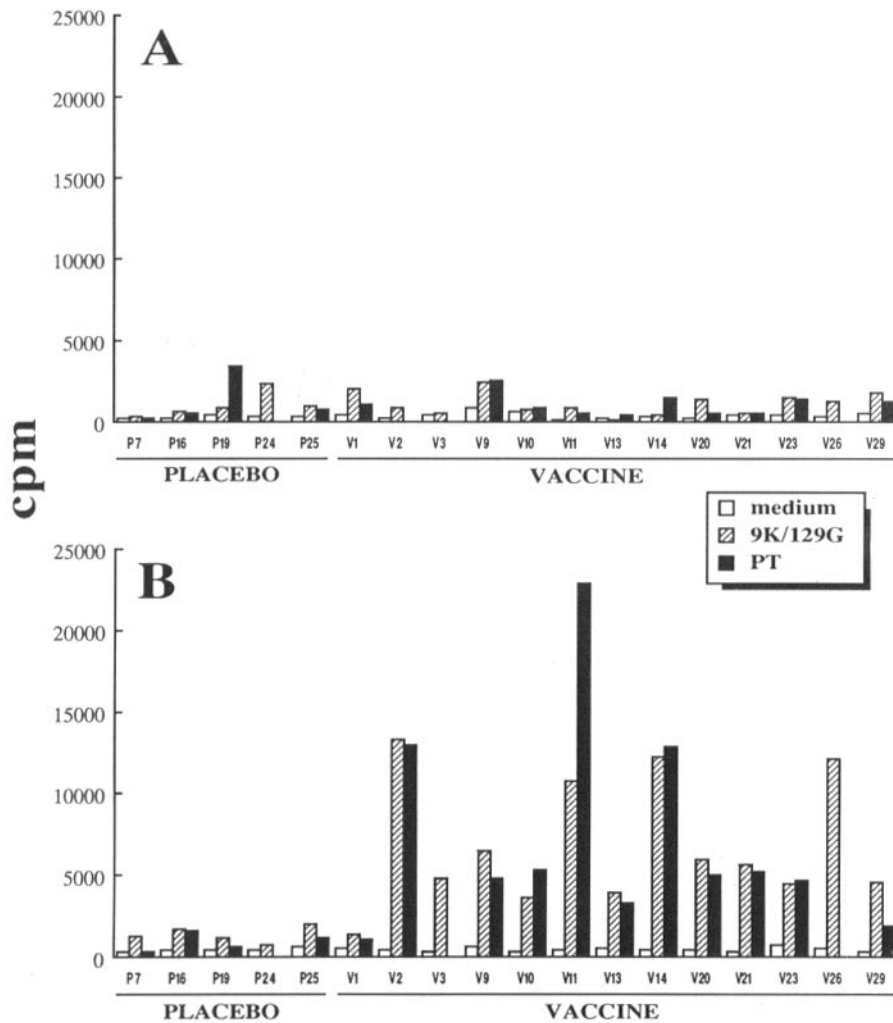


Figure 4. Proliferative responses to wild-type PT and PT-9K/129G in volunteers before (A) and 29 d after immunization (B). Proliferation on days +1 and +30 was not measured in all subjects but only in five placebos and 13 vaccinees that agreed to give larger samples of blood. On day +72, the cellular response, measured in two placebos and four vaccinees, did not show significant changes from day +30.

(CD23), were not increased by vaccination. Similarly, the proportion of T cells bearing γ/δ receptors, a cell subpopulation often reported to be increased during bacterial infections (27), remained unchanged. Based on this fact, it seems reasonable to conclude that PT-9K/129G does not induce in vivo any nonspecific lymphocyte activation. Very likely, the mitogenic activity of PT is just a side activity of receptor binding and does not play an active role in pathogenesis, as in the case of the staphylococcal toxins.

Humoral Response. One vaccine injection was capable of inducing high titers of antibodies specific for PT with strong neutralizing activity. Since the use of U.S. Reference Pertussis Antiserum in our ELISA and CHO tests allows reasonable comparison with previous studies, it can be concluded that 15 μg of PT-9K/129G induces a humoral response higher than that obtained using 50 or 25 μg of chemically detoxified PT (28, 29). This confirms in vivo with humans that genetic detoxification maintains the natural epitopes of the protein that are lost or altered with chemical detoxification (L. Nencioni, unpublished data). The second dose of vaccine did not cause any further increase of humoral responses, indicating that all adult volunteers had been previously exposed to *B. pertussis* and consequently gave a secondary response to the first immunization. Surprisingly, it was found that PT-9K/129G preparation, obtained by affinity chromatography according to Sekura (14), can induce in humans antibodies against FHA. A subsequent careful analysis by Western blot revealed that FHA traces (<0.1%) contaminated the vaccine preparation. Since the methods used throughout this study prove the specificity of the anti-PT response, this last result does not impair the overall conclusion about the effectiveness of the genetically inactivated toxoid, but rather, it provides further experimental evidence that PT-9K/129G can be included in a multi-antigenic vaccine composed of PT together with FHA and 69K, as suggested by several health authorities as the final acellular vaccine against whooping cough.

Cellular Immunity. Although antibodies are usually thought to play a major role in immunity against noninvasive bacterial infections, it is still a matter of debate which immune mechanisms are more important in protection against whooping cough. The recent finding in the Swedish clinical trial that the presence of anti-PT antibodies did not correlate with protection (7) may suggest that additional immune mechanisms such as cellular immunity may be important. We have previously studied the human T cell responses against *Bor-*

detella antigens (19, 20) and used the same techniques here to study the cellular immune response to vaccination with PT-9K/129G. Generally, we have observed that PT-9K/129G is able to stimulate cellular immunity in humans, as assessed in in vitro proliferation assays, and that the immunity induced is higher, but qualitatively similar, to that induced by PT during the disease (19, 20). In fact, the toxoid induces responses against both the A and the B oligomers, and increases also recognition of the whole *B. pertussis* cells. The lack of variation of the response against tetanus toxoid demonstrates the specificity of the immunity induced by the vaccine. Of particular interest was the finding that donor V2 showed high cellular immunity and negligible antibody titers. By conventional methods, this donor should be classified as a nonresponder. However, the fact that V2 possesses a strong proliferative response indicates that his immune system has acquired an immunity against PT. The same donor had cellular immunity but no antibodies against tetanus toxoid. This donor is in good health, and therefore represents an interesting subject to be further analyzed. Even if not so dramatic, the opposite result was obtained with donor V1, who had very high humoral responses and undetectable proliferation. The limited number of volunteers renders it difficult to understand how often these dichotomies between humoral and cellular responses can be observed. Nonetheless, these results show that measuring cellular immunity, in addition to antibody production, may add useful information for the evaluation of the protective immunity against whooping cough.

Conclusion. Although this study in human volunteers was only the first clinical step toward a new vaccine against whooping cough, the results obtained so far are very encouraging in terms of safety and immunogenicity for PT-9K/129G. Since the efficacy of PT in preventing whooping cough has already been established in the Swedish clinical trial (7), the presence of a molecule that eliminates all problems associated with chemical detoxification of PT suggests that PT-9K/129G should be the antigen of choice for future vaccines against whooping cough. These vaccines may contain either PT-9K/129G alone or combined with other antigens, such as FHA, 69K, or pili. More generally, it can be concluded that genetic manipulation can be efficiently used to modify the properties of natural molecules in order to make them suitable for human use, and that safe acellular pertussis vaccines are now feasible.

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