INFECTION AND IMMUNITY, May 1994, p. 1658–1661
0019-9567/94/$04.00+0

Vol. 62, No. 5

# Transgene-Encoded Antiphosphorylcholine ($T15^+$) Antibodies Protect CBA/N (*xid*) Mice against Infection with *Streptococcus pneumoniae* but Not *Trichinella spiralis*

PAK LEONG LIM,[1]* W. F. CHOY,[1] S. T. H. CHAN,[2] D. T. M. LEUNG,[3] AND S. S. M. NG[2]

*Clinical Immunology Unit, The Chinese University of Hong Kong,[1] and Department of Zoology[2] and Department of Physiology,[3] The University of Hong Kong, Hong Kong*

Received 8 June 1993/Returned for modification 8 December 1993/Accepted 28 January 1994

**Immunodeficient CBA/N (*xid*) mice are highly susceptible to *Streptococcus pneumoniae* infection. Previous studies indicated that this susceptibility may be attributed to the lack of antibodies to phosphorylcholine (PC) in the circulation of these animals. We now provide direct proof that when these mice are genetically manipulated to produce significant amounts of circulating anti-PC immunoglobulin G antibodies of the T15 idiotype, they can be protected against a lethal challenge with *S. pneumoniae*. Transgenic mice were also used to investigate whether the transgene-encoded antibodies could protect the animals against another PC-bearing microorganism, *Trichinella spiralis*; in this case, there was no protection. These results were further supported by experiments with CFW mice which had been immunized to produce high levels of anti-PC antibodies but which were found to be just as susceptible to *T. spiralis* infection as nonimmunized animals.**

CBA/N mice have a recessive X-linked immunodeficiency trait (*xid*) which affects their immune responses to carbohydrate antigens, including the phosphorylcholine (PC)-associated antigen found in *Streptococcus pneumoniae* (13). They have very little, if any, anti-PC antibodies in their circulation, as a result of which they succumb readily to *S. pneumoniae* infection (4). This bacterium bears abundant amounts of PC in its capsule and cell wall (2). On the other hand, immunologically normal mice, such as BALB/c mice, possess measurable levels of anti-PC antibodies and are better protected against the pneumococcus. Indeed, the serum of normal BALB/c mice can confer protection to CBA/N animals when given passively (4).

CBA/N mice provide a good model for various protection studies but are limited in that only passive immunization can be used, since the animals do not respond readily to many antigens, notably PC. There are problems associated with passive immunization; for example, the antibodies (especially those of the immunoglobulin M [IgM] class) can be denatured through processing, and the levels of circulating antibodies decline over time. We avoided these problems in the present study by using transgenic CBA/N mice which expressed modest levels of transgene-encoded anti-PC antibodies of the T15 idiotype in the circulation (9a). We showed that these animals were better protected against a pneumococcal challenge than animals not producing anti-PC antibodies.

We further exploited the transgenic CBA/N mouse system to determine whether anti-PC antibodies are protective against the nematode *Trichinella spiralis*. This intracellular nematode expresses vast amounts of PC in its (inner) cuticle (7), but the significance or protective value of this antigen remains unknown.

## MATERIALS AND METHODS

**Animals.** CBA/N mice, transgenic for an IgG2b antibody, were obtained by microinjection of mouse embryos with linearized plasmid g4R (9a). This 23.8-kb plasmid is similar to the IgM DNA plasmid described previously (9) except that the Cμ gene is replaced by mouse Cγ2b genomic DNA (*Eco*RI fragment) (12). Thus, in addition to the $C_H$ genes, g4R contains the $T15V_H$, $T15V_L$, and Cκ genes derived from the S107 myeloma cell line. Thus, the encoded protein is a $T15^+$, PC-binding IgG2b antibody.

Six-month-old transgenic mice (22 to 30 g) were used in this study. Transgene-negative littermates of these mice were used as controls.

Normal CFW (Swiss) mice were obtained originally from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Immunization of these animals with pneumococcal antigen was done by injecting 400 μg of alum-precipitated Pneumovax (Merck Sharp & Dohme, West Point, Pa.) in complete Freund's adjuvant intraperitoneally into each mouse. A booster dose (80 μg) of the alum-precipitated material was given intraperitoneally 1 month later.

**Antibody assays.** An enzyme-linked immunosorbent assay (ELISA) based on the direct binding of the test antibody to the nonsolubilized target antigen was used as described previously (10). The antigen used was a crude whole-worm extract (TSC) of *T. spiralis* muscle larvae (6). The test serum was incubated in antigen-coated microtiter plates (Immunon-2; Dynatech Laboratories, Alexandria, Va.) overnight at 4°C. Following washing, peroxidase-labelled goat anti-mouse IgG (whole molecule) (Sigma Chemical Co., St. Louis, Mo.) was added and incubated for 4 h at 37°C. Following development with substrate, the results were read with a Dynatech (MR 720) reader. Antibody concentrations were expressed in absolute units based on a standard or as titers based on an optical density cutoff of 0.2.

Western blot (immunoblot) analysis was performed as described previously (7) with TSC as the adsorbent. The assay was developed with a 1:74 serum dilution, biotin-labelled anti-mouse IgG (whole molecule) (1:2,000 dilution; Sigma), and extravidin-peroxidase (1:2,000; Sigma). *T. spiralis*-reactive monoclonal antibodies Ts2Ab and 7C2C5Ab were obtained as described previously (7).

**Pneumococcal challenge.** The method used previously for pneumococcal challenge (10) was followed. Freshly grown

* Corresponding author. Mailing address: Clinical Immunology Unit, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, N.T., Hong Kong.

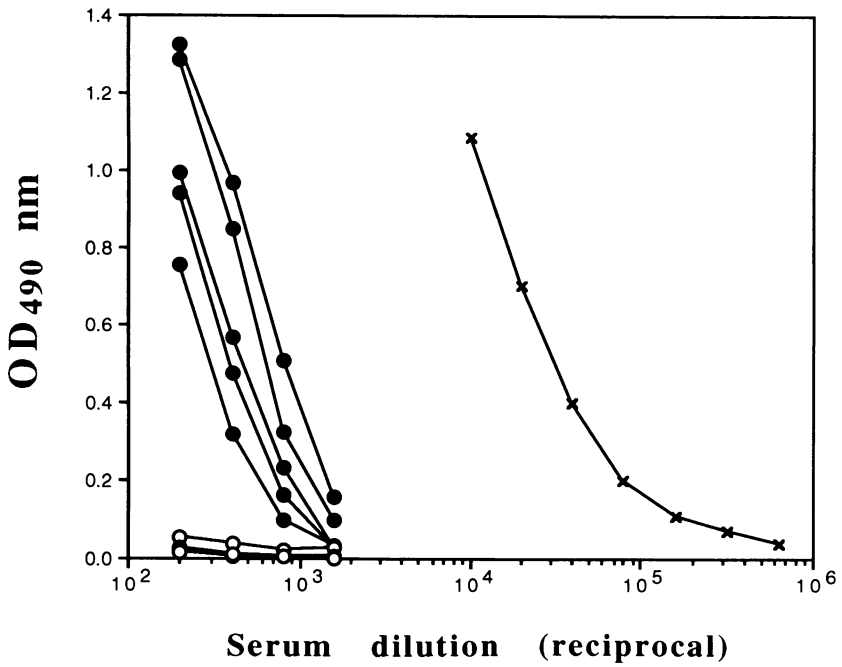


FIG. 1. ELISA binding curves for individual sera from transgenic (●) and control (○) mice. ×, Ts2Ab standard (ca. 0.6 mg/ml). $OD_{490}$, optical density at 490 nm.

*S. pneumoniae* type 3 (American Type Culture Collection strain) (590 ± 40 organisms) in 0.2 ml of serum broth was injected into the femoral vein of the animals. The mice were checked daily for survival.

***T. spiralis* challenge.** A total of 200 to 300 *T. spiralis* muscle larvae, freshly isolated from BALB/c mice (6), were administered orally to each mouse. One month later, the animals were sacrificed and the whole skinned, eviscerated carcass was homogenized, treated with 1% pepsin–10% HCl at 37°C overnight, and filtered over wire gauze (mesh size, 0.3 mm). The larvae present in the filtrate were washed, and samples (about 3%) of the larval suspension were enumerated with a dissecting microscope.

**Statistics.** A comparison of data between groups was done with the Student *t* test.

## RESULTS

**Pneumococcal challenge in transgenic CBA/N mice.** Five transgenic CBA/N mice (all female, *xid/xid*) and six transgene-negative siblings (all female) of these mice were used in the challenge experiment. The transgenic mice had significant levels (3 to 15 μg/ml) of anti-PC antibodies in the circulation, measured just prior to the experiment (Fig. 1). In contrast, the control animals had negligible amounts (<0.2 μg/ml) of anti-PC antibodies.

Following intravenous challenge of the mice with *S. pneumoniae* (590 organisms), two of the control animals died on day 2, and the remaining four died on day 3 (Fig. 2). In contrast, all of the transgenic animals survived through day 3, one died on day 4, another died on day 5, and the remaining three animals survived for more than 15 days (Fig. 2). Thus, the transgenic animals survived significantly ($P < 0.001$) longer than the control animals.

***T. spiralis* challenge in transgenic CBA/N mice.** Five transgenic CBA/N mice (all male) and five transgene-negative siblings (three male and two female) of these mice were used in the challenge experiment. While all of the transgenic animals had significant levels of anti-PC antibodies, as determined by an ELISA with various PC-associated antigens as the absorbents, none of the control mice did (data are not shown but are similar to those in Fig. 1). Sera from the transgenic mice were also reactive with a 39-kDa antigen present in TSC when analyzed by Western blotting (Fig. 3). One of the sera (lane B) showed considerably weaker activity than the others, although this was only marginally so in the ELISA findings (data not shown). In contrast, all of the control mouse sera were negative in the analysis. The same antigen was identified by a PC-binding monoclonal antibody (Ts2Ab), whereas other antigens were recognized by a non-PC-binding monoclonal antibody (7C2C5Ab).

One month following oral challenge of mice with 200 *T. spiralis* L1 larvae, there was no significant difference ($P = 0.14$) in larval burden between the transgenic (mean ± standard error, 11,437 ± 247 larvae; range, 9,862 to 13,429) and the nontransgenic (mean ± standard error, 9,680 ± 369 larvae; range, 7,921 to 12,054) groups.

***T. spiralis* challenge in PC-immunized CFW mice.** The study done to determine whether anti-PC antibodies are protective against *T. spiralis* was repeated with mice of the CFW strain which, unlike the CBA/N strain, can respond to the pneumococcal PC-associated antigen. The five mice immunized with this antigen showed a significantly ($P = 0.003$) higher level of antibodies (mean titer ± standard error, 1:1,488 ± 295; range, 1:600 to 1:2,400) to the *T. spiralis* antigen (mostly anti-PC antibodies) than did the five control, nonimmunized animals (mean titer ± standard error, 1:175 ± 81; range, 1:25 to 1:480).

When challenged orally with 300 *T. spiralis* L1 larvae, all mice in the study became infected. The larval burden determined at 1 month postchallenge was not significantly different ($P = 0.54$) between the immunized (mean ± standard error,

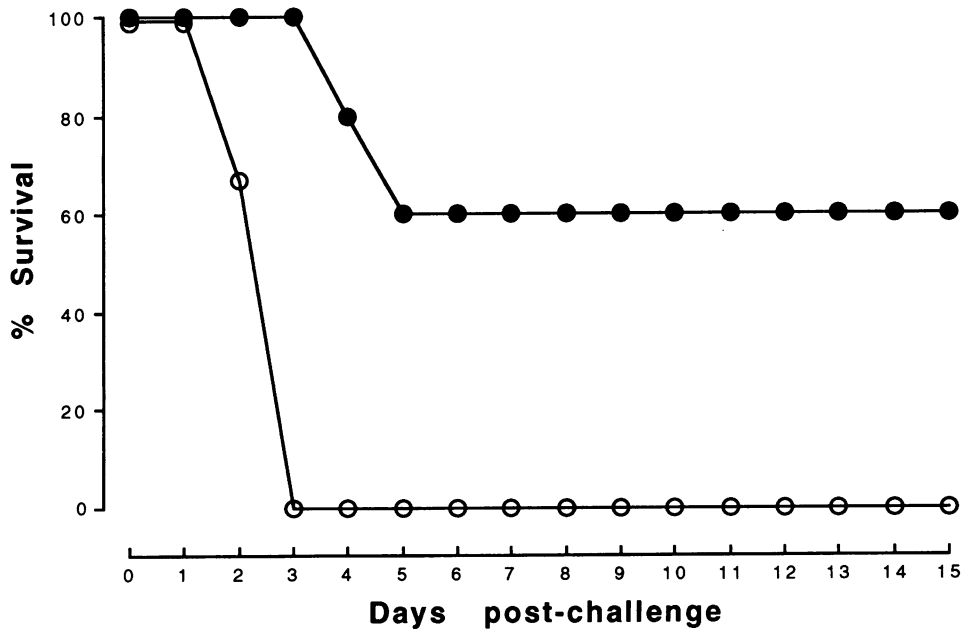


FIG. 2. Survival curves for transgenic (●) and control (○) CBA/N mice following challenge with *S. pneumoniae* on day 0.

29,083 ± 1,737 larvae; range, 11,766 to 34,522) and the control nonimmunized (mean ± standard error, 33,515 ± 2,169; range, 14,335 to 48,053) animals.

## DISCUSSION

This study demonstrates the possibility of genetically manipulating an animal to overcome its inherent inability to cope with particular infections. As far as we know, this is the first time that transgenic animals have been used for this purpose.

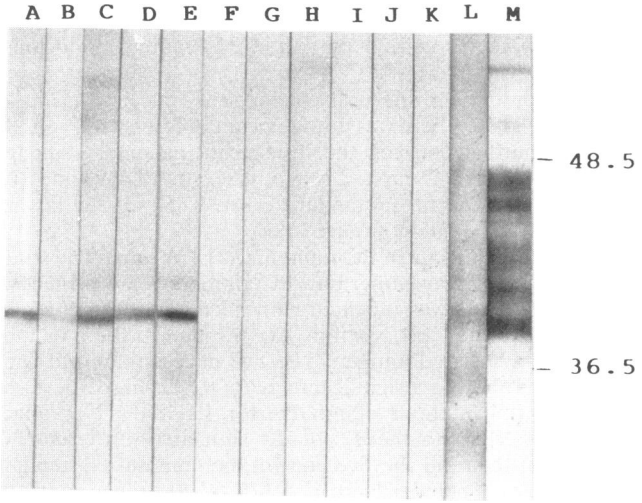


FIG. 3. Western blot results for individual sera from transgenic mice (lanes A to E) and control mice (lanes F to J) tested with TSC. Lane K, buffer control (no antibody); lane L, Ts2Ab; lane M, 7C2C5Ab. Numerical values denote molecular mass markers (in kilodaltons).

We have shown that *xid* CBA/N mice made to produce modest amounts (3 to 15 μg/ml) of $T15^+$ IgG anti-PC antibodies through the introduction of a transgene are significantly protected against a pneumococcal challenge (Fig. 2). This result confirms the specific role of anti-PC antibodies shown previously only in passive immunization studies with normal CBA/N animals (3, 4) and in the active immunization of normal CFW mice (10). The level of protection achieved in the transgenic animals was similar to that observed in normal CBA/N mice given 20 μg of $T15^+$ IgG1 hybridoma antibodies intraperitoneally 1 to 2 h prior to challenge (3).

Although the transgenic model may have no real advantage over passive transfer studies for examining resistance to pneumococci, it is of greater value in the study of chronic infections, such as those caused by *T. spiralis*. This parasite persists for long periods in the muscle of the host following its migration (and transformation) from the port of entry in the gut (5), and only in the transgenic model would constant exposure of the parasite to antibodies be ensured. Using such a system, we found that anti-PC antibodies were not protective against challenge with *T. spiralis*. This result is surprising in view of the abundance of PC in this organism (7, 11) and the fact that several other antigens have been shown to be protective (8, 14; see also reference 1). Although the number of animals used was perhaps small for a definitive analysis, the findings are supported by our active immunization experiments done with CFW mice. Moreover, we also obtained preliminary evidence that a $T15^+$ IgM anti-PC antibody (Ts2 [7]) given orally with *T. spiralis* larvae made no difference in infectivity in normal CBA/N mice (data not shown).

The difference in protection against the two organisms in transgenic mice may be explained by the fact that *S. pneumoniae* is an extracellular parasite, whereas the *T. spiralis* larva persists embedded in a "nurse cell" in the host and is protected by the highly calcified surroundings of the cell (5). Cell-mediated immunity may thus be more important in protection against a *T. spiralis* infection. It is possible, on the other hand,

that the blood-borne phase of the disease, involving the migration of the newborn larvae from the gut to the muscle, is amenable to antibody intervention, but at this stage of the cycle, the larvae do not express PC on their surface (11). It is also possible that, whereas the transgene-encoded antibody—a $T15^+$ IgG protein—is ideal for protection against the pneumococcus (3), other classes of antibodies (e.g., IgE) may be more appropriate for protection against the nematode. Alternatively, the amount of effective antibodies present in our animals was not sufficient for the nematode because of the fact that this organism is considerably larger than the pneumococcus.

## ACKNOWLEDGMENTS

We thank Jenny Mok for help in typing the manuscript.

We thank the University and Polytechnic Grants Committee of Hong Kong for support of this study.

## REFERENCES

1. **Appleton, J. A., R. F. Bell, W. Homan, and F. Van Knapen.** 1991. Consensus on *Trichinella spiralis* antigens and antibodies. Parasitol. Res. **7:**190–192.
2. **Bennett, L. G., and C. T. Bishop.** 1977. Structure of type 27 *Streptococcus pneumoniae* capsular polysaccharide. Can. J. Chem. **55:**8–16.
3. **Briles, D. E., C. Forman, S. Hudak, and J. L. Claflin.** 1982. The effects of idiotype on the ability of IgG1 antiphosphorylcholine antibodies to protect mice from fatal infection with *Streptococcus pneumoniae*. Eur. J. Immunol. **14:**1027–1030.
4. **Briles, D. E., M. Nahm, K. Schroer, J. Davie, P. Baker, J. Kearney, and R. Barletta.** 1981. Antiphosphocholine antibodies found in normal mouse serum are protective against intravenous infection with type 3 *Streptococcus pneumoniae*. J. Exp. Med. **153:**694–705.
5. **Campbell, W. C.** 1983. Trichinella and trichinosis. Plenum Press, New York.
6. **Choy, W. F., P. L. Lim, and M. H. Ng.** 1988. A comparison of immunological methods for the detection of *Trichinella spiralis* antigen. J. Immunol. Methods **113:**17–24.
7. **Choy, W. F., M. H. Ng, and P. L. Lim.** 1991. *Trichinella spiralis*: Light microscope monoclonal antibody localization and immunochemical characterization of phosphorylcholine and other antigens in the muscle larva. Exp. Parasitol. **73:**172–183.
8. **Gamble, H. R.** 1985. *Trichinella spiralis*: immunization of mice using monoclonal antibody-isolated antigens. Exp. Parasitol. **59:** 398–404.
9. **Guise, J. W., P. L. Lim, D. Yuan, and P. W. Tucker.** 1988. Alternative expression of secreted and membrane forms of immunoglobulin μ-chain is regulated by transcriptional termination in stable plasmacytoma transfectants. J. Immunol. **140:**3988–3994.

9a. **Lim, P. L., S. T. H. Chan, D. T. M. Leung, S. S. M. Ng, and T. T. Loh.** Mol. Immunol., in press.

10. **Lim, P. L., and W. F. Choy.** 1990. A thymus-independent (type 1) phosphorylcholine antigen isolated from *Trichinella spiralis* protects mice against pneumococcal infection. Immunology **69:**443–448.
11. **Takahashi, Y., W. Homan, and P. L. Lim.** 1993. Ultrastructural localisation of the phosphorylcholine-associated antigen in *Trichinella spiralis*. J. Parasitol. **79:**604–609.
12. **Tucker, P. W., K. B. Marcu, N. Newell, J. Richards, and F. R. Blattner.** 1979. Sequence of the cloned gene for the constant region of murine Cγ2b immunoglobulin heavy chain. Science **206:**1303–1306.
13. **Wicker, L. S., and I. Scher.** 1986. X-linked immune deficiency (*xid*) of CBA/N mice. Curr. Top. Microbiol. Immunol. **124:**87–101.
14. **Zhu, D., and R. G. Bell.** 1990. *Trichinella spiralis*: murine strain variation in response to monoclonally defined, protective, non-stage-specific antigens. Exp. Parasitol. **70:**330–343.