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Monoclonal Idiotope Vaccine Against *Streptococcus pneumoniae* Infection

Abstract. *A monoclonal anti-idiotope antibody coupled to a carrier protein was used to immunize BALB/c mice against a lethal* Streptococcus pneumoniae *infection. Vaccinated mice developed a high titer of antibody to phosphorylcholine, which is known to protect against infection with* Streptococcus pneumoniae. *Measurement of the median lethal dose of the bacteria indicated that anti-idiotope immunization significantly increased the resistance of BALB/c mice to the bacterial challenge. Antibody to an idiotope can thus be used as an antigen substitute for the induction of protective immunity.*

The network hypothesis of Jerne (*1*) defines the immune system as a web of interacting idiotopes. Idiotopic markers are found on the variable regions of both immunoglobulins and T-cell receptors (*2–4*). Inherent in the network theory is the postulate that external or nominal antigens are mimicked by idiotope structures. If these internal antigens could be exploited as antigenic structures, an alternative set of antigens would be available for vaccine preparations that could complement conventional vaccines.

Idiotope vaccines offer distinct advantages over conventional vaccines derived from nominal antigens, which often require elaborate and expensive purification. Idiotope vaccines can be easily prepared by producing hybridoma anti-idiotope antibodies and coupling them to immunogenic carrier molecules. Biological advantages arise from the fact that idiotope antigens exist in a different molecular environment from that of nominal antigens, which allows an idiotope vaccine to stimulate silent or tolerized immune clones (*5, 6*). The latter point bears on the issue of tumor antigens to which an acquired state of tolerance often exists. If T cells are "educated" by idiotypes and internal images (*7, 8*), the antibody to the idiotope may elicit effective T-cell help through associative recognition.

We now report the use of a modified monoclonal anti-idiotope antibody as a vaccine against a virulent bacterial infection in BALB/c mice. Our approach is based on data showing that the TEPC 15 (T15) idiotype, an antibody specific for phosphorylcholine (PC), is effective in protecting mice against a lethal *Streptococcus pneumoniae* infection (*9, 10*). We selected the binding site–specific hybridoma antibody 4C11 from a set of monoclonal A/He anti-T15 antibodies prepared previously (*11*). The 4C11 antibody was coupled to keyhole limpet hemocyanin (KLH) with glutaraldehyde (*12*).

Normal BALB/c mice were used in these experiments because this strain produces a dominant T15 idiotype (*13*). The mice were injected twice during a 1-month period (at 2-week intervals) with doses (100 μg each) of KLH, PC-KLH, 4C11, 4C11-KLH, 315, or 315-KLH (315 is a nonrelated idiotype). One week after the second injection, the mice were bled and their sera tested for antibodies to PC and for the T15 idiotype by an ELISA (enzyme-linked immunosorbent assay) and by radioimmunoassay. The results (Table 1) showed that only PC-KLH and 4C11-KLH caused significant elevations of antibody to PC. Used alone as an immunogen, 4C11 also increased the level of antibody to PC though not to that achieved by 4C11-KLH or PC-KLH.

Type 3 virulent *S. pneumoniae* (American Type Culture Collection) was grown on Trypticase soy agar supplemented with 10 percent sterile sheep red blood cells. Normal and immunized BALB/c mice were challenged with 10^4, 10^5, 10^6, 10^7, or 10^8 *S. pneumoniae* administered intravenously; these doses were based on the results of preliminary LD_{50} (median lethal dose) experiments. Deaths were recorded on a daily basis for 7 days (Table 2). The LD_{50} dose for normal, unprimed BALB/c mice is approximately 4×10^5 bacteria, a relatively large number (*10*) resulting from the naturally high concentration of T15 idiotype in the

Table 1. Serum response of BALB/c mice to nominal and internal antigens. The mice received intraperitoneal injections (100 μg each) of the immunogens listed suspended in complete Freund adjuvant; 2 weeks later they received a booster injection of the same immunogen in incomplete Freund adjuvant. Antibodies to PC were determined by an ELISA; concentrations (± standard error of the mean) were measured by comparison to a standard. The amount of T15 idiotype (>92 percent in all cases) in the antibody to PC was determined by means of ^{125}I-labeled monoclonal anti-T15 antibody in a radioimmunoassay; n = about 20 mice per group.

Immunogen	α-PC in serum (μg/ml)
Control	35.6 ± 6
315	25 ± 2
315-KLH	70 ± 15
KLH	81 ± 27
PC-KLH	8800 ± 2500
4C11	542 ± 184
4C11-KLH	5072 ± 675

Table 2. Protection of BALB/c mice against *Streptococcus pneumoniae* infection by nominal and idiotope vaccines. Mice were immunized as described (see Table 1) and challenged with serial dilutions (tenfold) of *S. pneumoniae* administered intravenously 5 days after the second injection of immunogen. Deaths were recorded daily for 7 days. The LD_{50} was estimated as described (*19*). Log (protection) indicates the relative amount of protection for immunized animals compared to normal unprimed controls [log (protection) = 0]. The number of survivors is recorded for the 10^6 *S. pneumoniae* challenge dose; S.E.M., standard error of the mean.

Immunogen	LD_{50} of *S. pneumoniae* (± S.E.M.)*	Log (protection)	Survivors (No.)
Control	3.8×10^5 (1.7×10^5)	0	9 of 28
KLH	1.9×10^5 (1.6×10^5)	−0.3	3 of 10
PC-KLH	1.9×10^7 (1.4×10^7)	+1.7	7 of 9
315-KLH	1.1×10^6 (1.4×10^6)	+0.4	6 of 11
4C11	1.4×10^6 (5×10^5)	+0.6	10 of 19
4C11-KLH	2.3×10^7 (6.6×10^6)	+1.8†	30 of 32

*Standard errors were calculated on the basis of $n = 3$. †$P < 0.01$ compared to control (t-test).

serum. Statistical analysis of the LD_{50} data showed that KLH, 315-KLH, and 4C11 did not significantly increase the resistance of the mice to pneumococcal infection. The LD_{50} for mice primed with PC-KLH and 4C11-KLH, however, was almost 100 times greater than that for normal mice, indicating that an idiotope vaccine can be as effective in eliciting protection as the conventional vaccine based on nominal antigen (PC-KLH) (*14*). The high titers of antibody to PC in mice primed with PC-KLH and 4C11-KLH (Table 1) correlated with the increased protection seen in the LD_{50} experiments.

These results show that a monoclonal anti-idiotope vaccine is effective in protecting mice against a lethal bacterial infection. The functional significance of an idiotope-based vaccine has been suggested (*15–17*) and was demonstrated against African trypanosomiasis (*18*). However, that work differed from ours in that an allogeneic idiotypic antiserum preparation was used as an immunogen.

The antibody to the idiotope alone was not effective in protecting against *S. pneumoniae* but had to be coupled to a protein carrier. Presumably, T-helper cells needed for the serum antibody response are activated by the conjugate of idiotope antibody and carrier. The relative inefficiency of the internal idiotope alone in inducing protection can be predicted from the immune network hypothesis, which dictates that the internal antigens must remain silent for homeostasis of the immune system.

Internal antigen images provide the means to construct novel vaccines that may prove superior to conventional ones. The most promising application of idiotope vaccines is in the immunotherapy of tumors, which is hampered by the unavailability of pure tumor antigens and the preexisting tolerance to tumor-associated antigens.

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Essential Role of Insulin in Transcription of the Rat 25,000 Molecular Weight Casein Gene

Abstract. *Insulin is essential for the accumulation of rat casein messenger RNA (mRNA) in the presence of glucocorticoid and prolactin. The accumulation of certain mRNA's in other tissues has also been linked to insulin action. The present study shows that the accumulation effect on the 25,000 molecular weight rat casein mRNA does not reflect stabilization of the transcript by insulin. Rather, insulin is essential for its synthesis in the presence of glucocorticoid and prolactin.*

In a review of studies on the effects of insulin on RNA (*1*), most of the reports showed that the levels of certain proteins are elevated by insulin and that these effects are prevented by actinomycin D, an inhibitor of RNA synthesis. Insulin-dependent gene expression has also been shown to be related to changes in the accumulation of the corresponding messenger RNA's (mRNA's) (*2–6*). The murine mammary gland is a model useful for studying the role of insulin in relation to mRNA. Insulin (*7*), glucocorticoid (*8*), and prolactin (*7*) are essential for the accumulation of casein mRNA [molecular weight, 25,000 (25K)] in rat mammary explants. Although insulin-like growth factor II (IGF II) [multiplication stimulating activity (*9*)] and IGF I [somatomedin C (*10*)] can maintain the tissue in the synthetic medium as well as insulin, they do not mimic effectively the action of insulin either on casein synthesis or on the accumulation of casein mRNA. This suggests that the effect of insulin on casein gene expression is hormone-specific and does not involve interaction of the hormone with receptors for IGF I or II.

Increased accumulation of mRNA can result from stabilization of the transcript or an increased rate of transcription or both. In experiments to determine the effect of insulin on these effectors of the accumulation of rat 25K casein mRNA, rat mammary explants were cultured in Medium 199 to which four agents were added in various combinations. Serum was added to all the systems because it can maintain the tissue in the absence of insulin (*7*). In those instances in which it was of interest to operate in the absence of serum-derived prolactin, antiserum to prolactin was used. Antiserum to insulin was added when it was of interest to operate in the absence of serum-derived

Table 1. Effect of insulin (I) and prolactin (P) on the accumulation of rat 25K casein mRNA. Mammary explants from rats at day 12 of pregnancy were cultured in 2.5 ml of Medium 199 as described (*17*). All tissue was first cultured with lamb serum (20 percent) and cortisol (50 ng/ml) for 2 days (SF). Culture was then continued with added prolactin (1 μg/ml) or insulin (10 ng/ml) or both for up to 3 days more. At the start of the second culture period, antiserum to insulin (1 μl per dish) was added to all systems to which insulin had not been added, and antiserum to ovine prolactin (2 μl per dish) was added to all systems to which prolactin had not been added. In all cases, the SF medium was treated with the appropriate antiserum for 12 hours before tests. A 1-μl sample of insulin antiserum precipitated 30 ng of insulin, and a 2-μl sample of prolactin antiserum precipitated 150 ng of prolactin. Total RNA was isolated from the explants by the guanidinium–cesium chloride method (*18*). The 25K casein mRNA component was determined by the DBM disk method (*8*). Total RNA (1 μg) bound to DBM disks was hybridized with nick-translated ^{32}P-labeled p530–25K casein DNA (480,000 count/min) at 42°C for 18 hours. Each value represents the mean of triplicate determinations ± standard error. Comparable results were obtained in three similar experiments. Zero time indicates the freshly isolated tissue; N.D., not detectable.

Conditions	Day	Hybridization*
Zero time		7183 ± 227
SF	2	174 ± 8
	5	N.D.
SF → SFP	5	26 ± 14
SF → SFI	5	N.D.
SF → SFPI	3	404 ± 43
	4	768 ± 98
	5	2293 ± 31

*Amount of ^{32}P-labeled p530–25K casein DNA hybridized, expressed as counts per minute per microgram of RNA.