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Naturally Acquired Immunity to Tetanus Toxin in an Isolated Community

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Literature on natural immunity to tetanus is scarce. We examined antitetanus antibody levels with the enzyme-linked immunosorbent assay in 200 people living in an isolated community and clarified the influence of age and sex on immunity. In 197 subjects, antitoxin antibodies were measured. No sex differences were noted, and 30% had protective levels (above 0.01 IU/ml). The percentage of those considered protected was age dependent.

When the literature on tetanus and immunity to the disease is reviewed, there appears to be almost a consensus that natural immunity to tetanus does not exist (6) and that only active immunization guarantees with certainty protection against the clinical manifestations of the disease (1, 3). However, in a growing number of recent publications, data have been reported on naturally acquired immunity (2, 11, 12) in humans who had not received vaccinations. To date no evidence has been available which might explain how these people acquired such antibodies.

We had the unique opportunity to explore this problem in a group of Jewish immigrants (named Falashees) from Ethiopia and in particular to investigate the effect of age and sex on the tetanus antibody titer. In Ethiopia, health services are notoriously poor. It has been estimated that there is about one physician per 100,000 people. The very small ancient Jewish community in Ethiopia has suffered from prolonged persecution and cultural isolation. This community has been deprived of the poor health services in the country, and according to our information, none of the subjects included in our study had ever been attended by a physician, let alone received any injections during their lifetimes.

Immediately after their arrival in Israel the new immigrants from Ethiopia were brought to an absorption center. All were medically examined by health authorities. A venous blood sample was taken from each individual, serum separated, and kept frozen at −20°C. A total of 200 serum samples were randomly chosen by one of the authors and subjected to tetanus antibody examination. Of the samples 102 were from males and 98 were from females. The range of ages varied from 1 to 82 years (Table 1).

Serum antibodies were determined by an enzyme-linked immunosorbent assay (ELISA) which has recently been described (4, 5, 8, 9) and found to be very sensitive even with low concentrations of antibody (5, 9). Briefly, tetanus toxoid (Rafa Laboratories, Jerusalem, Israel) was attached to the inner well surfaces of disposable microtitration plates (Nunc Immunoplates; Nunc, Roskilde, Denmark) by incubation for 24 h at 4°C. Serum samples diluted 1/200 and antibody controls were then added, and the plates were incubated for 1 h at 37°C. The wells were then washed and incubated overnight at 4°C with alkaline phosphatase-conjugated goat antiserum immunoglobulin G (Miles Yeda, Rehovot, Israel). Phosphatase substrate (Sigma Chemical Co., Tel Aviv, Israel) was then added. The reaction was allowed to develop at room temperature and then stopped by the addition of 20% NaOH. Each titration was performed in duplicate. Results of optical density measurement were compared with results of a standard antitetanus preparation (Tetaglobin; Rafa Laboratories). A standard curve was constructed by using the commercial antiserum containing 250 IU/ml. All readings were performed by the same investigator (S.R.).

The results for the study subjects are given in Table 1. The range of positive ELISA readings was between 0.002 and 0.023 IU/ml. Of the 200 subjects admitted to our study, 61 were found to have antitoxin antibodies of more than 0.01 IU/ml (considered well protected); of them 32 were males and 29 were females. A total of 139 subjects were found to have less than 0.01 IU/ml (thus "unprotected"); of them 70 were males and 69 were females. No differences were therefore noted between the sexes. The average age of those with protective levels was 36.07 + 0.33 years, compared with 28.59 + 0.12 in the unprotected group. Of the 139 unprotected subjects, only 3 were found to lack any antitoxin antibodies. Thus, out of the 200 subjects, 197 were found to have some measurable level of antibody.

The chi-square test revealed high significance ($P < 0.05$) when protective levels were compared among three age groups: 1 to 10 years, 11 to 60 years, and over 60 years of age, irrespective of sex. There was a rapid increase in protective levels in young adults aged 11 to 20 years. Statistical significance of less than 0.05 was demonstrated only between those less than 50 and those over 50 years of age. When geometric mean concentrations for men and women in each age group were compared, no differences were observed.

Several years before the discovery and introduction of tetanus toxoid as a vaccine, Tenbroeck and Bauer (10) were able to detect tetanus bacteria in the stool and antitoxin in the blood of one-third of a group of citizens of Peking, China. Since then, several publications have appeared describing natural immunity in small isolated populations (11, 12). There is enough evidence today to challenge the axiomatic statement that only active immunization is the key to disease prevention.

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Table 1. Results of blood serum antitoxin titration (ELISA)

Age group (yrs)	Males			Females			Total		
	No. tested	% Protected[a]	Titer (mean ± SD)	No. tested	% Protected[a]	Titer (mean ± SD)	No. tested	% Protected[a]	Titer (mean ± SD)
1–10	7	0	0.007 ± 0.001	15	13	0.005 ± 0.002	22	9	0.006 ± 0.001
11–20	25	36	0.008 ± 0.001	30	30	0.008 ± 0.001	55	33	0.008 ± 0.001
21–30	23	22	0.007 ± 0.002	15	27	0.008 ± 0.002	38	24	0.008 ± 0.002
31–40	23	30	0.008 ± 0.001	8	25	0.007 ± 0.002	31	29	0.007 ± 0.001
41–50	10	40	0.008 ± 0.002	13	15	0.006 ± 0.001	23	26	0.007 ± 0.001
51–60	3	33	0.009 ± 0.001	9	33	0.009 ± 0.001	12	33	0.009 ± 0.001
61+	11	55	0.009 ± 0.002	8	75	0.012 ± 0.001	19	63	0.011 ± 0.001
Total	102	31	0.007 ± 0.002	98	29	0.007 ± 0.002	200	30	0.007 ± 0.002

[a] Antitoxin titers over 0.01 IU/ml were considered to be protective.

Veronesi et al. (12) describe in their preliminary report a population of only 58 adults. In a later publication (11) they describe another 57 people living on an isolated island, again a population too small for specific demographic description. We believe our data contribute much needed information about the prevalence of immunity and the age at which immunity is acquired, since we had the opportunity to study a larger population and use the very sensitive ELISA technique. We could better identify the population that might have been thought to lack antibodies if different, less sensitive techniques were used. We found that over 98% of our study group had measurable antibodies. In only three subjects were no antibodies demonstrated. Two of these were females less than 2 years of age, and the third was a 30-year-old male. Increasing titers were noted with greater age.

Although only 30% exhibited more than the accepted protective titer of 0.01 IU/ml (7), the percentage of those considered protected was age dependent, increasing substantially from 10% in the first decade to an average of 29% in the 11- to 60-year-old group to 63% in the group over 60 years of age. Natural immunity to tetanus is gained, as in many other diseases, through adequate, repeated, and prolonged antigenic stimulation that sensitizes the immune system. The opportunities for achieving immunity increase with age, and this is well reflected in our data. It may be hypothesized that repeated exposure over many years to the tetanus bacilli (perhaps by growth of the bacilli in the tissue or by colonization of the digestive system), with relatively small amounts of toxin produced, may ultimately both immunize and provide a booster to the individual. Since the tetanus bacilli are ubiquitous, it is not unexpected that in primitive cultures where women and men perform agricultural tasks resulting in repeated exposure to soil, the tetanus immunity is not sex dependent.

Because the disease is still taking a heavy toll in developing countries, killing between 500,000 and 1,000,000 children each year, one should note that it is imperative to implement immediate immunization in those countries. In light of the results of our study, the questions that still have to be answered are whether those afflicted by the disease were adequately challenged (e.g., children) and whether the antibody levels achieved by this natural immunity are sufficient to protect one from the disease.

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LITERATURE CITED

1. **Ajjan, N., N. Souvras, J. Duhamel, and M. Rey.** 1981. A study of tetanus immunization among older subjects, p. 185–197. *In* Proceedings of the Sixth International Conference on Tetanus. Fondation Merieux, Lyon, France.
2. **Dastur, F. D., V. P. Awatramani, S. K. Dixit, J. A. D'Sa, N. D. Cooverji, and M. P. Anand.** 1981. Reponsè to single dose of tetanus vaccine in subjects with naturally acquired tetanus antitoxin. Lancet **i**:219–221.
3. **Eckmann, L.** 1963. Tetanus, prophylaxis and therapy. Crune & Stratton, Inc., New York.
4. **Fillastre, C., B. Bizzini, M. T. Fayet, J. C. Vincent-Falquet, M. Huet, J. C. Artus, and J. L. Sartou.** 1981. Titrage des anticorps antitetaniques par differentes techniques, p. 467–476. *In* Proceedings of the Sixth International Conference On Tetanus. Fondation Merieux, Lyon, France.
5. **Galazka, A.** 1981. Serological methods for detection and quantification of tetanus antibodies, p. 307–329. *In* Proceedings of the Sixth International Conference on Tetanus. Fondation Merieux, Lyon, France.
6. **Immunization Practices Advisory Committee.** 1981. Diphtheria, tetanus and pertussis—guidelines for vaccine prophylaxis and other preventive measures. Ann. Intern. Med. **95**:723–728.
7. **McComb, J. A.** 1964. Prophylactic dose of homologous tetanus antitoxin. N. Engl. J. Med. **270**:175–178.
8. **Nieminen, S., M. Viljanen, and M. Nurmi.** 1979. Tetanus morbidity and immunity in Finland. Acta Chir. Scand. **145**: 379–383.
9. **Stiffler-Rosenberg, G., and H. Fey.** 1977. Messung von Tetanus Antitoxin mit dem Enzyme linked immunosorbent assay. Schweiz. Med. Wochenschr. **107**:1101–1104.
10. **Tenbroeck, C., and J. H. Bauer.** 1923. Studies on the relation of tetanus bacilli in the digestive tract to tetanus antitoxin in the blood. J. Exp. Med. **37**:470–479.
11. **Veronesi, R., B. Bizzini, R. Focaccia, A. L. Coscina, C. C. Mazza, M. T. Focaccia, F. Carraro, and M. N. Honnigman.** 1983. Naturally acquired antibodies to tetanus toxin in humans and animals from Galapagos Islands. J. Infect. Dis. **147**:308–311.
12. **Veronesi, R., H. Cecin, A. Correa, J. Tavares, C. Morales, and O. J. Bertoldo.** 1975. New concepts on tetanus immunization: naturally acquired immunity. J. Hyg. Epidemiol. Microbiol. Immunol. **19**:126–134.