TITRATIONS OF ANTIBODIES AGAINST α-HAEMOLYSIN AND THE COMPONENTS OF STAPYHLOCOCCAL LEUCOCIDIN IN HUMAN SUBJECTS FOLLOWING IMMUNIZATION*

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Staphylococcus pyogenes is perhaps more versatile in its offensive capabilities than any other known bacterial pathogen. It possesses a varied array of physiologically active metabolites, some inimical to its hosts. Staphylococcal cells also, unlike streptococci and pneumococci, are capable of resisting prompt destruction within phagocytes (Rogers and Tompsett, 1952; Goodman, Moore and Baker, 1956; Kapral and Shayegani, 1959; Rogers and Melly, 1960; Mackaness, 1960; Shayegani and Kapral, 1962). In addition, staphylococci seem to have an almost unique facility to adapt to inimical environments, such as those containing antibiotics.

The hypothesis is certainly defensible, therefore, that under different conditions offensive capabilities of the staphylococcus may become critical. In acute septicaemia, for instance, it may be assumed that exotoxins are of primary importance and their neutralization critical for survival. In localized recurrent infection, such as chronic furunculosis, on the other hand, the capacity to resist intracellular destruction may be determinative.

The toxoids currently employed for immunization against staphylococcal infection are prepared on the assumption that staphylotoxin (α-toxin) is the factor of primary importance in pathogenesis, and its corresponding anti-toxin in resistance. Literature cited previously (Gladstone, Mudd, Hochstein and Lenhart, 1962) suggests that this premise may be a gross oversimplification. In particular, neglect of leucocidin and antileucocidin may be a serious deficiency. We have, therefore, undertaken to investigate as systematically as practicable actual responses to representative agents in current use for increasing specific resistance to staphylococcal infection. The present communication will be limited to responses in terms of antibodies to α-toxin and to Panton-Valentine leucocidin. Antibacterial immunity and related questions will be reserved for later consideration.

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MATERIALS AND METHODS

Divasta, Institut Pasteur.—This is a purified toxoid, fortified by 6×10^9 staphylococcal cells per ml. The staphylococcal cells represent the Cowan-Christie international serotypes (Pillet, Berrod, Gossey and Orta, 1956). For the Divasta we are indebted to Dr. A. Bonnefoi and Dr. J. Pillet of the Institut Pasteur, Paris and Garches, France.

Staphylococcus toxoid Sclavo (d'Antona, 1958).—For this toxoid we are indebted to Dr. D. d'Antona and Dr. A. Cinotti of the Istituto Sieroterapico Sclavo, Siena, Italy. Dr. d'Antona reported in 1958 that $1{\cdot}5 \times 10^4$ l. of toxoid Sclavo had been administered to human subjects in Italy alone.

Staphylococcus toxoid, Connaught Laboratories.—This was an experimental lot containing α and β toxoid, supplied through the courtesy of Dr. F. J. Wilson and Dr. J. M. Corkill, of the Connaught Medical Research Laboratories, Toronto, Canada.

Staphylococcus toxoid, Lederle.—This toxoid was courteously supplied to Drs. A. M. Kligman and Howard I. Maibach of the Department of Dermatology, University of Pennsylvania.

Staphylococcus polyvalent somatic antigen vaccine (Greenberg, Cooper and Healy, 1961).

Staphage, Delmont Laboratories.—Lysate of a strain of staphylococcus of phage group 1 plus staphylococcal bacteriophage. Supplied through the courtesy of Mr. Charles E. Lincoln, of Delmont Laboratories, Swarthmore, Pennsylvania.

Administration of these immunizing agents was according to the schedules recommended by the producing laboratories. Immunization was by the courtesy of those whose cooperation was acknowledges previously (Gladstone, *et al.*, 1962).

Titrations.—Antibodies neutralising α-haemolysin and the F and S components of Panton-Valentine leucocidin were titrated as described previously (Gladstone, *et al.*, 1962). Agglutinins were also titrated, following the growth pattern procedure of Boger, Frankel and Gavin (1960).

The populations to which immunizing agents were administered are described below.

RESULTS

United States Veterans Administration Hospital, Philadelphia

The first population given an immunizing agent was composed of members of the medical staff of the Veterans Hospital. Staphylococcus toxoid Sclavo (Siena) or a placebo were administered by code to alternate individuals on a randomized basis. Titrations were performed without knowledge of the code. Although this population was too small to be treated statistically, the rises in anti-α-haemolysin titres occurred in all subjects given toxoid and distinguished correctly those who received toxoid from those who received placebo (Table I). Changes in antibodies against the leucocidin components were of questionable significance.

In a later experience in the Veterans Hospital, personnel were given Connaught Laboratories' toxoid. Again significant increases in anti-α-haemolysin but not against the components of leucocidin occurred (Table I).

State Hospital, Milledgeville, Georgia

Toxoid Sclavo (Siena), Divasta and a placebo were administered by code in a large static population where their influence on infection, carrier rate and immunological status could be closely observed. A preliminary survey led to the selection of a group of 396 female epileptic psychotic patients. Infection was sporadic. The Siena toxoid and Divasta and a placebo were administered as coded preparations to groups of these patients. Clinical details are reported by Vogel, McCroan and Mudd, 1962).

The present report will be limited to titrations for anti-α-haemolysin and anti-leucocidin components, before and after immunization, on samples of approxi-

mately 20 patients in each of the 3 categories. The results are given in Tables II and III. Six months following immunization a recall course of immunization was given. The results appear in Table IV.

TABLE I.—*Titres of V.A. Hospital Personnel Immunized with Siena and Connaught Toxoids*

Immunizing agent	Subject	Anti-α-haemolysin (u./ml.) Before	Anti-α-haemolysin (u./ml.) After	Antileucocidin F (u./ml.) Before	Antileucocidin F (u./ml.) After	Antileucocidin S (u./ml.) Before	Antileucocidin S (u./ml.) After
Siena toxoid	1	0·5	2·1	0·3	0·6	0·4	0·8
	2	1·2	8·0	0·3	1·6	0·3	0·8
	3*	1·2	24·0	8·0	22·5	7·8	14·4
	4	0·8	9·9	0·8	10·0	1·0	5·8
	5	0·7	6·0	1·6	4·0	2·5	4·0
	6	0·4	4·0	0·2	1·3	0·3	0·5
Connaught toxoid	7	0·8	12·8	<1·0	2·5	1·0	3·2
	8	0·8	4·0	1·0	<1·0	0·5	1·3
	9	1·8	6·4	4·0	4·0	4·0	12·0
	10	1·6	3·2	0·5	1·2	<0·3	<0·3
	11	2·0	14·4	4·0	8·0	2·0	6·4
	12	0·8	2·4	<0·4	0·6	6·4	0·5

* Chronic tonsillitis; tonsillectomy March 1, 1960; immunization July 7–August 5, 1960.

TABLE II.—*Psychotic Patients—Anti-α Toxin Titres (Mean Values in International Units)*

	Placebo (u./ml.)	Siena toxoid (u./ml.)	Divasta (u./ml.
Before immunization	1·5 ± 0·8	1·7 ± 0·9	1·8 ± 1·0
After immunization	1·7 ± 1·2	11·5 ± 8·3	8·2 ± 6·6
Probability of difference being due to sampling	0·25–0·10	<0·005	<0·005

Significance of differences of mean differences: Siena—placebo, probability <0·005; Divasta—placebo, probability <0·005; Siena—Divasta, probability 0·25–0·10.

TABLE III.—*Psychotic Patients—Anti-Panton-Valentine Leucocidin Titres in Provisional Units per ml.*

	Placebo F Component	Placebo S Component	Siena toxoid F Component	Siena toxoid S Component	Divasta F Component	Divasta S Component
Before immunization	2·6; S.D. = 2·9	2·3; S.D. = 1·8	2·4; S.D. = 2·4	2·3; S.D. = 1·8	3·9; S.D. = 2·6	3·9; S.D. = 4·1
After immunization	2·9; S.D. = 3·0	3·0; S.D. = 2·0	4·2; S.D. = 3·4	4·4; S.D. = 4·0	3·8; S.D. = 2·1	3·2; S.D. = 2·6
Probability of difference being due to sampling	0·25–0·10	0·025–0·01	0·025–0·01	<0·005	>0·50	0·25–0·10

Significance of differences of mean differences: Siena—placebo, F, probability 0·05–0·025; S, probability 0·05± 0·025. Divasta—placebo, F, probability >0·50; S, probability 0·05–0·025 (decrease).

TABLE IV.—*Psychotic Patients—Anti-α Toxin Titres (Mean Values in International Units)*

	Placebo (u./ml.)	Siena toxoid (u./ml.)	Divasta (u./ml.)
Before immunization (BI)	1·7 ± 0·9	1·8 ± 0·9	1·8 ± 1·0
After immunization (AI)	2·2 ± 1·6	12·1 ± 8·9	8·8 ± 7·0
After recall (AR)	1·4 ± 0·7	5·2 ± 2·2	4·6 ± 3·0

Significance of differences of mean differences::

Placebo —	BI *v.* AR, probability,	0·025–0·10
	AI *v.* AR, „	0·10–0·05
Siena —	BI *v.* AR, probability,	0·005 (increase)
	AI *v.* AR, „	0·005 (decrease)
Divasta —	BI *v.* AR, probability,	0·005 (increase)
	AI *v.* AR, „	0·025–0·01 (decrease)

It is clear that both the Siena toxoid and Divasta caused significant increases in anti-α-haemolysin. The titres must have decreased some months later, as indicated in the literature (d'Antona, 1958), and were regained only partially by the recall injections (Table IV). Response in terms of antileucocidin was lacking or minimal (Table III).

State Colony, Woodbine, New Jersey

Divasta, Lederle toxoid, polyvalent somatic antigen vaccine and a placebo were administered under code to groups of 150 subjects each in a total population of 1,250 feeble-minded or retarded persons.

The infection rate was followed during the 8 months preceding immunization (May 1 to December 31, 1960), and for the 4 months from January 1 to May 1, 1961. Immunization was started and the first blood sample was drawn early in January, 1961. The final blood sample was taken late in April, 1961.

The infection rate in this population was extremely high before immunization (Gladstone *et al.*, 1962), and high during immunization, a fact which may well account for the statistically significant increase in mean titres in the placebo group. The increases in mean titres of anti-α-haemolysin in the groups receiving Divasta and Lederle toxoid were significantly greater than the increase in the placebo group (Table V). The increase in mean anti-α-haemolysin titre in the

TABLE V.—*Patients at Woodbine Colony—Anti-α-haemolysin Titres*

	Placebo (u./ml.)	Divasta (u./ml.)	Lederle toxoid (u./ml.)	Greenberg vaccine (u./ml.
Before immunization	1·2; S.D.= 0·8	1·5; S.D.= 0·9	1·2; S.D.= 0·7	1·3; S.D.= 1·0
After immunization	2·1; S.D.= 1·4	5·4; S.D.= 2·8	3·9; S.D.= 2·0	1·6; S.D.= 1·2
Probability of difference being due to sampling	<0·005	<0·005	<0·005	<0·01–0·025

Significance of differences of mean differences: Divasta—placebo, probability <0·005; Lederle—placebo, probability <0·005; Divasta (Milledgeville)—Divasta (Woodbine), probability, 0·10–0·05.

group receiving polyvalent somatic antigen was less than the increase in the placebo group, despite a lower infection rate in the placebo group. The lack of

antitoxic response to this vaccine is in agreement with the statement of Greenberg *et al.* (1961) that the method of manufacture and control tests " preclude the possibility of the presence of toxins or toxoids ".

There were no significant responses to immunization with respect to anti-leucocidin (Table VI) with the possible exception of a slight response to Lederle toxoid. In view of the high infection rate, interpretation of this slight increase is uncertain.

TABLE VI.—*Patients at Woodbine Colony—Anti-Panton-Valentine Leucocidin Titres in Provisional Units per ml.*

Fast Component—Antileucocidin

	Placebo	Divasta	Lederle	Greenberg
Before immunization	5·4; S.D.= 7·2	3·4; S.D.= 4·1	4·8; S.D.= 4·6	6·3; S.D.= 8·4
After immunization	5·0; S.D.= 5·4	3·9; S.D.= 4·9	5·8; S.D.= 4·1	5·2; S.D.= 7·2
Probability of difference being due to sampling	>0·50	0·1–0·2	0·25–0·10	0·20–0·10

Divasta—placebo, probability, 0·25–0·30
Lederle—placebo, probability, between 0·10 and 0·20
Divasta (Milledgeville)—Divasta (Woodbine), probability 0·4–0·5

Slow Component—Antileucocidin

	Placebo	Divasta	Lederle	Greenberg
Before immunization	5·0; S.D.= 3·9	5·6; S.D.= 3·9	5·7; S.D.= 3·6	7·0; S.D.= 8·0
After immunization	5·1; S.D.= 3·6	5·3; S.D.= 3·9	7·9; S.D.= 4·4	6·2; S.D.= 7·5
Probability of difference being due to sampling	>0·50	0·25–0·20	<0·005	0·20–0·10

Divasta—placebo, probability, 0·25–0·30
Lederle—placebo, probability, 0·005–0·01
Divasta (Milledgeville)—Divasta (Woodbine), probability, 0·5–0·6

St. Christopher's Hospital—Philadelphia

Nine children suffering from fibrocystic disease of the pancreas were given a course of injections of Connaught toxoid by Dr. Nancy Huang, with the hope of increasing their specific resistance. Titrations of blood samples taken before and after the course of injections are set out in Table VIII. It is clear that the anti-

TABLE VII.—*Titres of Patients with Fibrocystic Disease of Pancreas, St. Christopher's Hospital, Immunized with Connaught Toxoid*

Patient	Anti-α-haemolysin		Antileucocidin F		Antileucocidin S	
	Before	After	Before	After	Before	After
O'R	2·0	5·8	<0·4	1·0	0·3	0·3
SF	6·0	24·0	6·0	6·0	2·6	1·4
McE	4·5	6·8	<0·4	<0·4	<0·1	<0·1
CC	4·5	2·0	100	100	80	60
McG	1·0	20·0	<0·4	<0·4	1·2	1·2
DP	3·0	12·5	5·0	6·0	10·0	10·0
MJ	0·9	7·0	<0·4	<0·4	0·6	1·3
GC	2·9	7·0	2·0	1·2	4·0	4·0
RS	0·8	7·5	<2·0	0·4	1·2	2·6

Anti-α-haemolysin: Mean before immunization 2·8; S.D. = 1·9
Mean after immunization 12·3; S.D. = 7·1

α-haemolysin titres were significantly increased but the titres of anti-F and anti-S leucocidin were not.

Norristown State Hospital

A series of patients were given injections of Staphage as part of a therapeutic program for chronic furunculosis (Boger *et al.*, 1960). Titrations for anti-α-haemolysin of some 15 sera showed no significant change. Antileucocidin titres were as follows :

Mean anti-F leucocidin			before immunization		3·7 ; S.D.= 4·9
,,	,,	,,	after	,,	4·1 ; S.D.= 6·1
Mean anti-S leucocidin			before immunization		2·2 ; S.D.= 2·6
,,	,,	,,	after	,,	3·0 ; S.D.= 4·3

DISCUSSION

We are acutely appreciative of the numerous reports from many countries of the clinical usefulness of α-toxoid. Competent clinical observers and their associates in the laboratory have with few exceptions been convinced of the benefits obtained in properly chosen cases with judiciously administered toxoid (Mudd, 1960). The subject has been exhaustively reviewed by d'Antona (1958).

The fact that the toxoids investigated do not elicit antileucocidin, we regard as an unfortunate consequence of the fact that existing international and national regulations regarding staphylococcal immunizing agents require response in terms of anti-α-haemolysin only but include no specifications regarding antileucocidin. In view of the important work of English investigators and in particular of Johanovsky and his associates, (cited by Gladstone *et al.*, 1962), we believe that thorough investigation of the consequences of complementing existing immunizing agents with leucocidin toxoid is an imperative need. This we propose to undertake ourselves, and we hope others may do so.

SUMMARY

Standard staphylococcal toxoids, *i.e.* Institut Pasteur " Divasta ", Sclavo toxoid (Siena), Connaught Laboratories toxoid, and Lederle toxoid have been administered under controlled conditions to human populations and the serological responses determined. The groups receiving immunizing agents included healthy hospital personnel, inmates of two mental hospitals with some endemic staphylococcal infections, inmates of an institution for the feeble minded with very high endemic staphylococcal infection rate, and patients with fibrocystic disease of the pancreas. Anti-α-haemolysin was regularly elicited by all toxoids. None of the toxoids elicited significant responses in terms of antibodies against the fast and slow components of Panton-Valentine leucocidin.

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