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Immunological studies in nasal carriers of staphylococci

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Introduction

The major reservoir for coagulase positive staphylococci in man is the anterior nose [4, 9]. A variety of studies have demonstrated that human subjects tend either to be consistent carriers of coagulase positive staphylococci or to be consistent non-carriers over a prolonged period of time. Although bacterial interference may play a role in the maintenance of the carrier status, this does not seem to satisfactorily explain the marked differences in the propensity of individuals to be either carriers or non-carriers.

The following studies were undertaken to determine if there were differences in the immunological reactions to staphylococcal antigens in subjects who were carriers of coagulase positive staphylococci as compared to subjects who were not.

Material and methods

Carriers of coagulase positive staphylococci were defined as individuals who had at least two consecutive positive cultures by quantitative nasal methods [11] at least one week apart. Non-carriers were similarly defined as those who had no coagulase positive staphylococci isolated during the same period of time.

The methods used in this laboratory for the following assays have been published: muramidase and beta-glucuronidase [2], measurement of histamine [6], release of histamine on exposure to antigens [7], preparation of white cell extracts [6], determination of antibodies against teichoic acids [3]. The methods used for measuring phagocytosis and killing of staphylococci have been published by *Quie* et al. [8].

Procedure

The procedure for human leukocyte chemotaxis was as follows: Leukocytes were collected from fresh, human, venous blood by the dextran sedimentation method. The cells were washed twice in tris A (tris, NaCl, KCl, 0.3 mg% albumin pH 7.5) and resuspended in tris A plus Ca, Mg. and 5% heat inactivated autologous serum at 1×10^7 cells/ml. A dialyzed and lyophilized culture filtrate of *E. coli* was resuspended in tris A CM with 5% heat inactivated human serum at 20 mg dry wt/ml and diluted serially.

Chemotaxis chambers were taken from the method of *Cornely* [1] and consisted of 3–4 dram plastic snap-cap vials, 13 mm diameter lucite tubes and 13 mm Millipore

membranes with 3μ pore size. Membranes were glued to one end of the lucite tubes which were inserted through holes in the vial caps into the vials. Vials contained 4 ml of test material and the lucite tubes contained 1 ml of cell suspension. Chemotaxis was carried out at 37°C for 3 hours; the membranes were removed, fixed and stained with Ehrlich's hematoxylin. The membranes were mounted in resin and examined under 400× magnification. Cells which had migrated the entire membrane thickness were counted in five random fields and averaged. Only the cells in the first plane of focus on the test material side of the filter membrane were counted.

Results

The migration of white cells to the source of infection is one important host-defense mechanism against staphylococcal disease. The response of white cells to a chemotaxis factor was measured by incorporating an extract of *E. coli* in a modified Boyden chamber. From 17 to 64 white cells per high power field migrated from the system in three hours with no significant differences in the chemotaxis response of white cells from nasal carriers of coagulase positive staphylococci as compared to cells from non-carriers (Fig. 1).

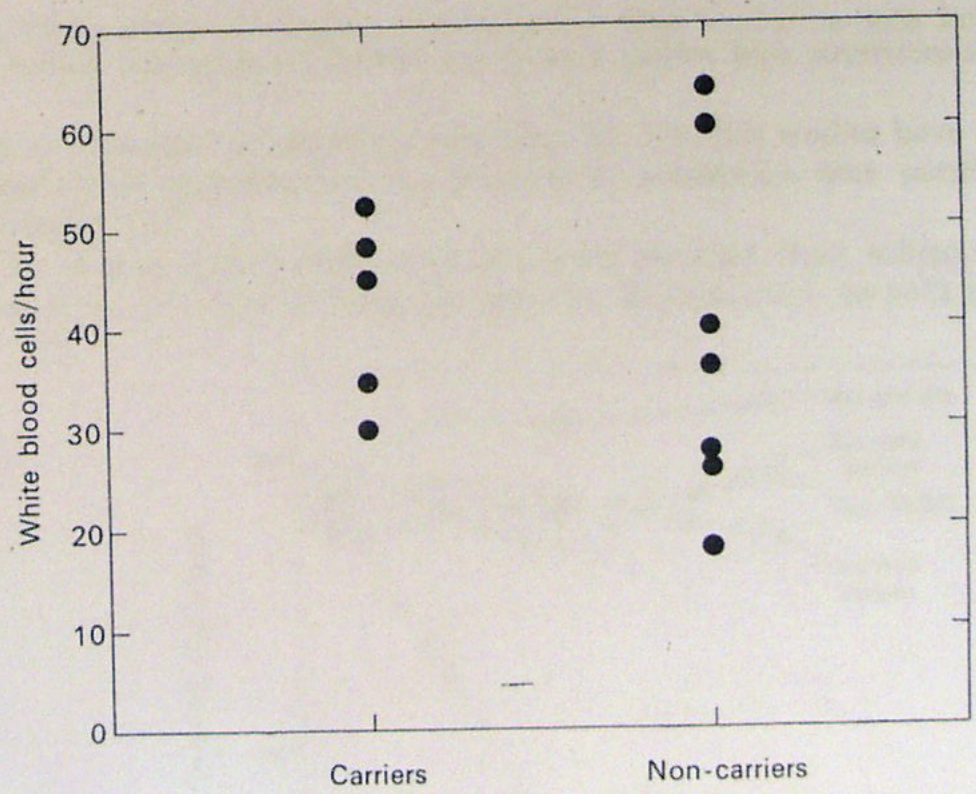

Fig. 1. A comparison of the response of white cells from carriers and non-carriers to chemotaxis.

Phagocytosis and intracellular killing of coagulase positive staphylococci is another important defense mechanism. In most subjects, maximal phagocytosis and killing of coagulase positive staphylococci occurs when human white cells are incubated in unheated sera. Heating the sera at 56°C for one hour markedly impairs or eliminates the ability of most sera to promote phagocytosis and killing. In a few subjects, particularly those with documented severe staphylococcal diseases, heat stable opsonins are present so that there are minimal differences between the opsonic activity of

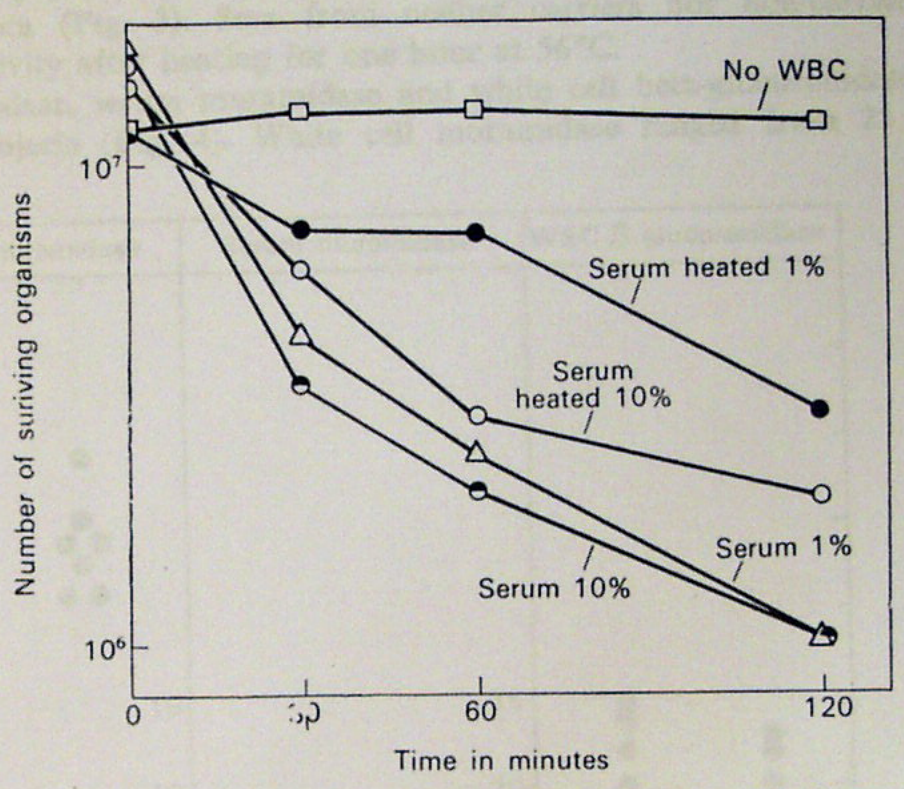

Fig. 2. The number of staphylococci surviving after incubation with human white cells in heated and unheated human sera from a patient with staphylococcal infection.

heated as compared to unheated sera (Fig. 2). Previous studies have demonstrated that the heat stable opsonins can be removed by absorption with purified staphylococcal mucopeptide [5].

In the present study, WBC and sera were obtained from subjects who were either carriers or non-carriers of coagulase positive staphylococci. In both groups of subjects,

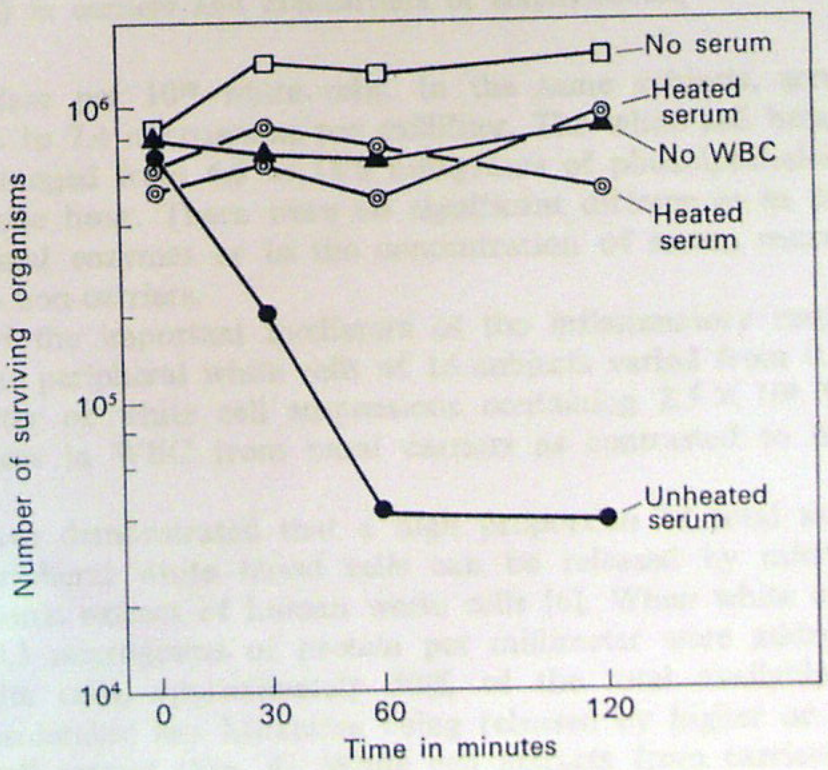

Fig. 3. The survival of staphylococci following incubation with human white cells and sera from uninfected carriers or non-carriers of staphylococci. This illustration is typical of the response in sera and white cells from 7 non-carriers and from 5 carriers.

over 90% of the staphylococci were killed within one hour of incubation with WBC in unheated sera (Fig. 3). Sera from neither carriers nor non-carriers had significant opsonic activity after heating for one hour at 56°C.

White cell muramidase, serum muramidase and white cell beta-glucuronidase were assayed in twelve subjects (Fig. 4). White cell muramidase ranged from 22 to 38

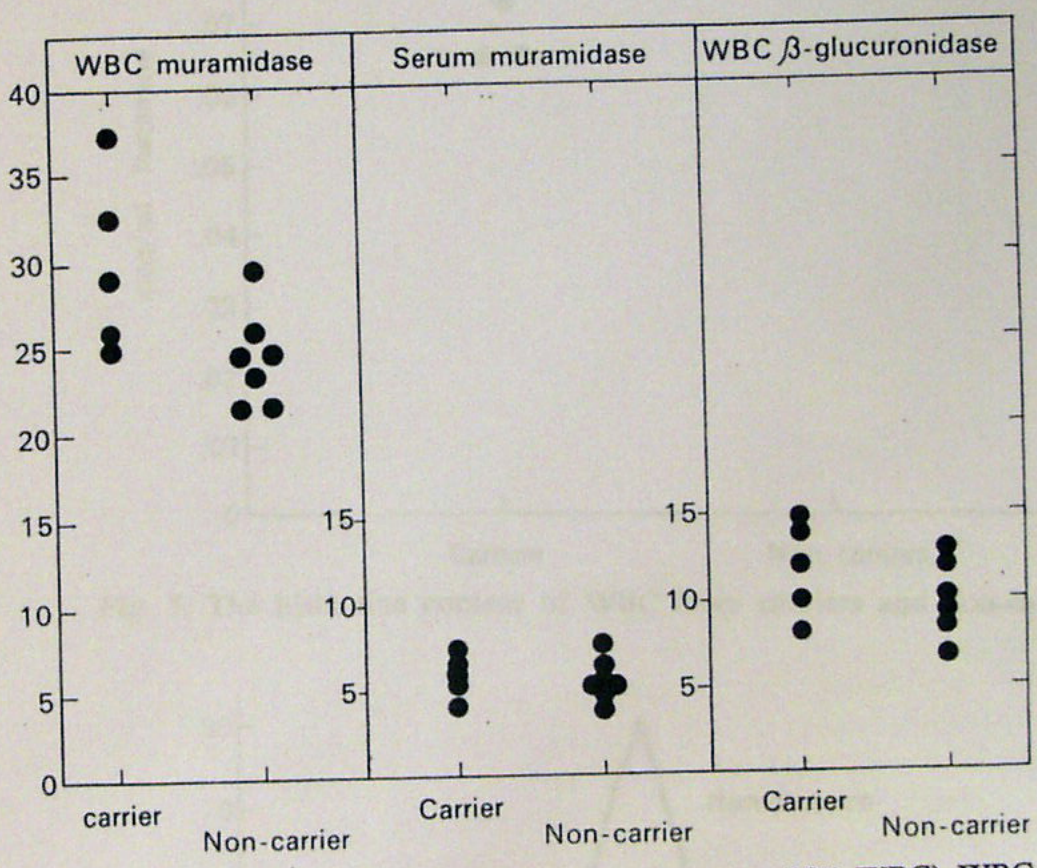

Fig. 4. A comparison of the content of WBC muramidase (mg/10^{10} WBC) WBC beta-glucuronidase (mg/phenolphthalein liberated per hour per 10^{10} WBC) and serum muramidase (γ/ml) in carriers and non-carriers of staphylococci.

milligrams of muramidase per 10^{10} white cells. In the same subjects, serum muramidase ranged from 4.6 to 7.4 micrograms per milliliter. The white cell beta-glucuronidase in these subjects ranged from 6.5 to 14.6 milligrams of phenolphthalein released by 10^{10} white cells in one hour. There were no significant differences in the concentration of these lysosomal enzymes or in the concentration of serum muramidase in carriers as compared to non-carriers.

Histamine is one of the important mediators of the inflammatory response. The histamine content of the peripheral white cells of 16 subjects varied from 0.66 to 0.89 micrograms per milliliter of white cell suspensions containing 2.5×10^6 WBC with no significant differences in WBC from nasal carriers as contrasted to non-carriers (Fig. 5).

Previous studies have demonstrated that a high proportion of total available histamine in human peripheral white blood cells can be released by micrograms of protein from an ultrasonic extract of human white cells [6]. When white cell extracts at concentrations of 0.3 micrograms of protein per millimeter were added to intact human peripheral white cells, approximately 50% of the total available histamine was released with considerable less histamine being released by higher or lower concentrations of white cell extract (Fig. 6). White cell extracts from carriers and non-carriers of coagulase positive staphylococci all released histamine maximally at a 1 to

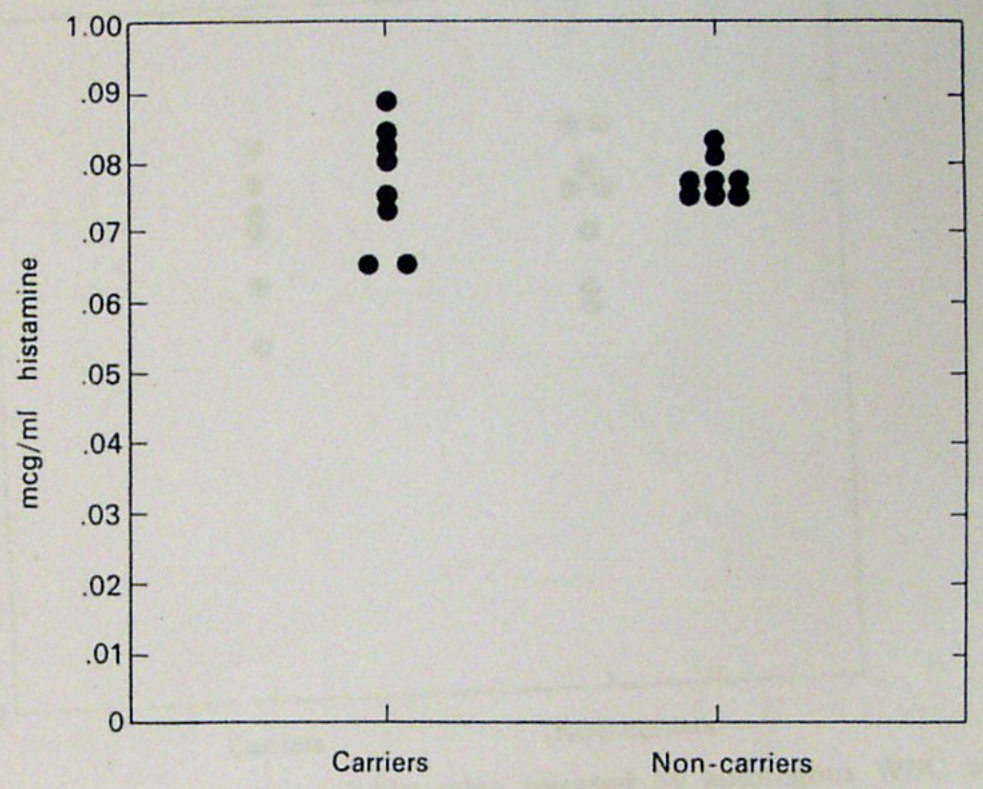

Fig. 5. The histamine content of WBC from carriers and non-carriers.

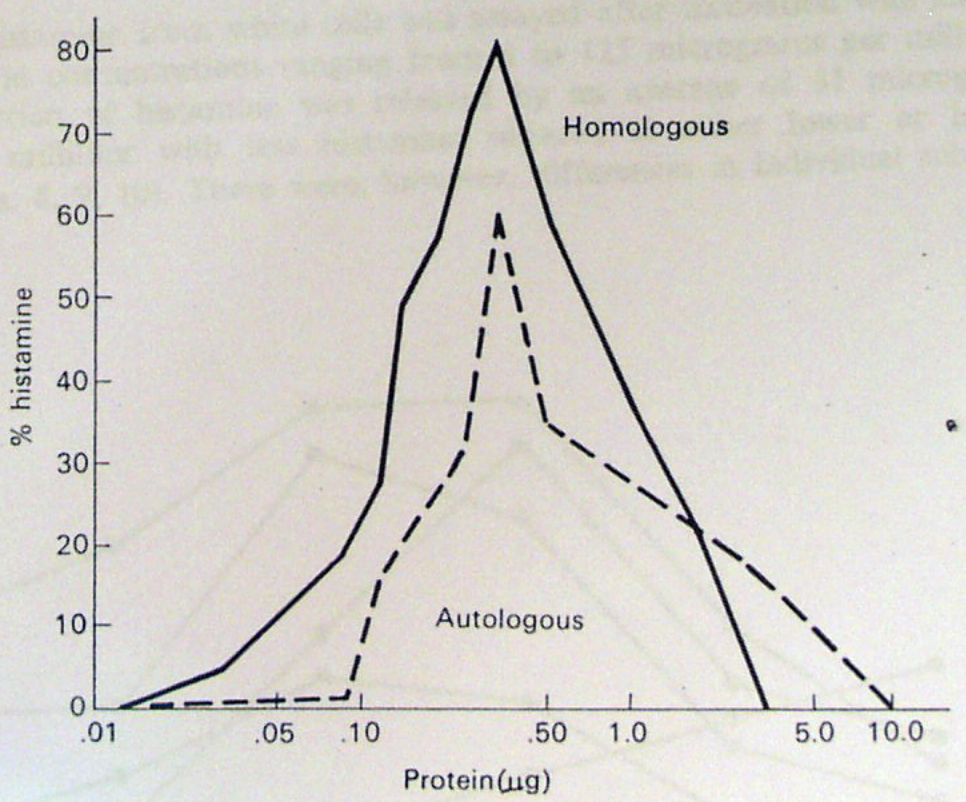

Fig. 6. The per cent of histamine released from peripheral human WBC after incubation with small amounts of extracts of autologous or homologous WBC.

3,000 dilution of extracts containing 0.96 to 1.7 mg protein per ml. The proportion of histamine released varied from 40 to 58 per cent (Fig. 7). There was no difference in the proportion of histamine released by extract of homologous white cells from intact WBC of carriers of the coagulase positive staphylococci as compared to the release of white cell extracts from non-carriers.

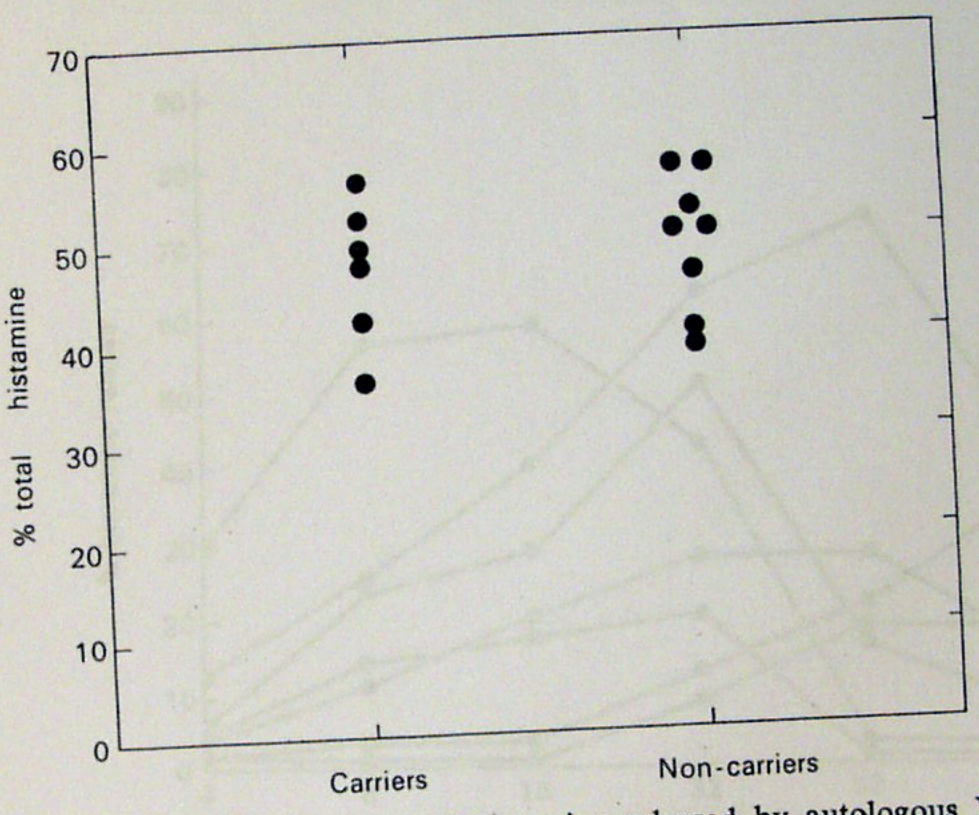

Fig. 7. A comparison of the proportion of histamine released by autologous WBC extract from WBC of carriers and non-carriers.

The release of histamine from white cells was assayed after incubation with staphylococcal protein A at concentrations ranging from 4 to 125 micrograms per milliliter. The highest proportion of histamine was released by an average of 31 micrograms of protein A per milliliter with less histamine released at either lower or higher concentrations (Figs. 8, 9, 10). There were, however, differences in individual subjects;

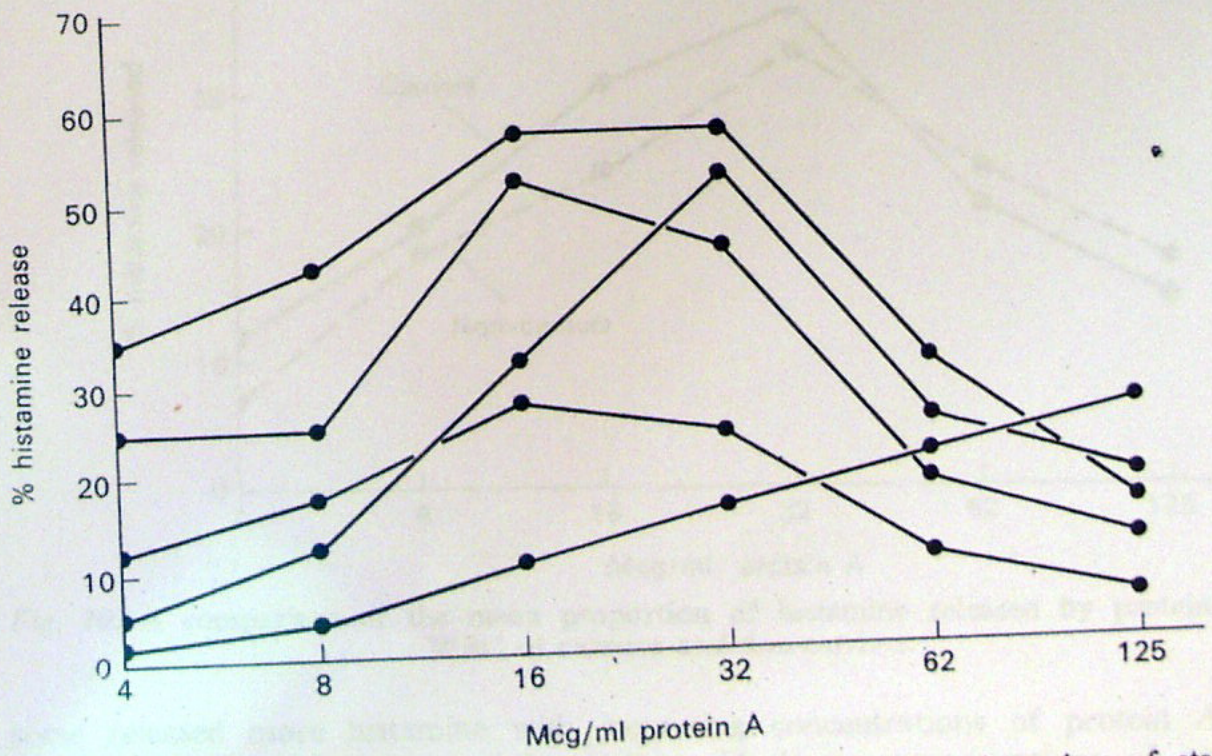

Fig. 8. The proportion of histamine released from peripheral WBC of carriers of staphylococci after incubation with protein A.

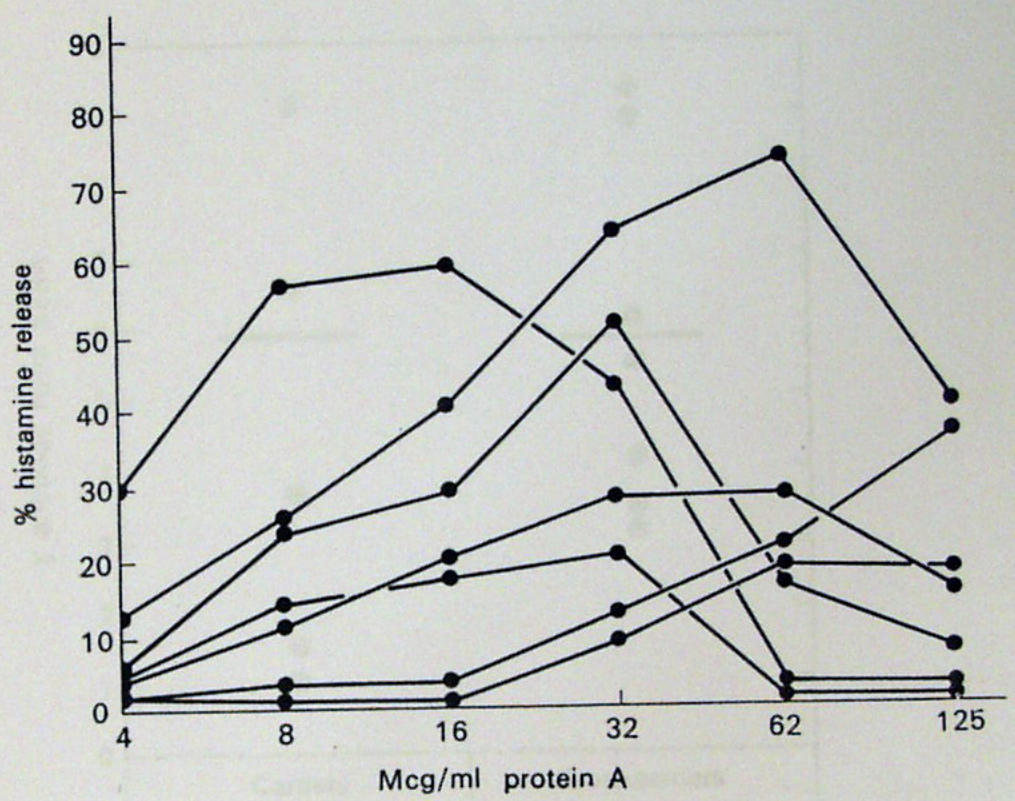

Fig. 9. The proportion of histamine released from peripheral WBC of non-carriers of staphylococci after incubation with protein A.

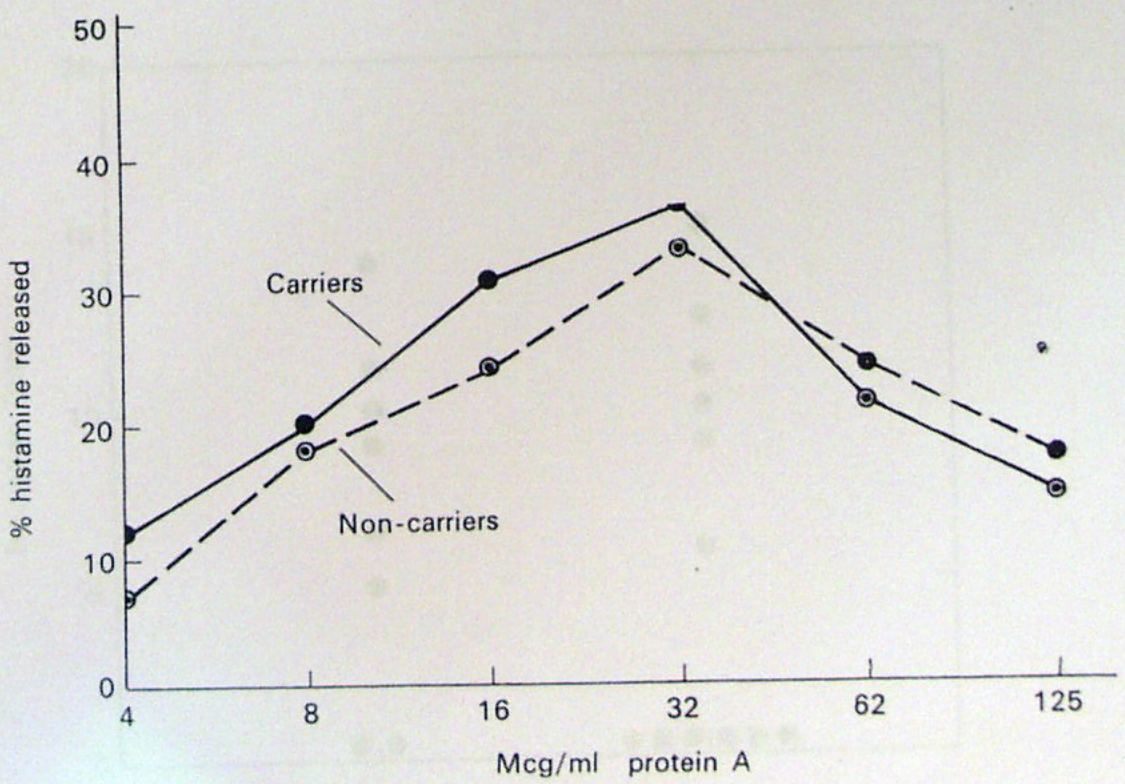

Fig. 10. A comparison of the mean proportion of histamine released by protein A from WBC of carriers and non-carriers.

some released more histamine with increasing concentrations of protein A, others released the largest amount of histamine with lower concentrations of protein A. There were no differences in the mean proportion of histamine released by carriers as compared to non-carriers.

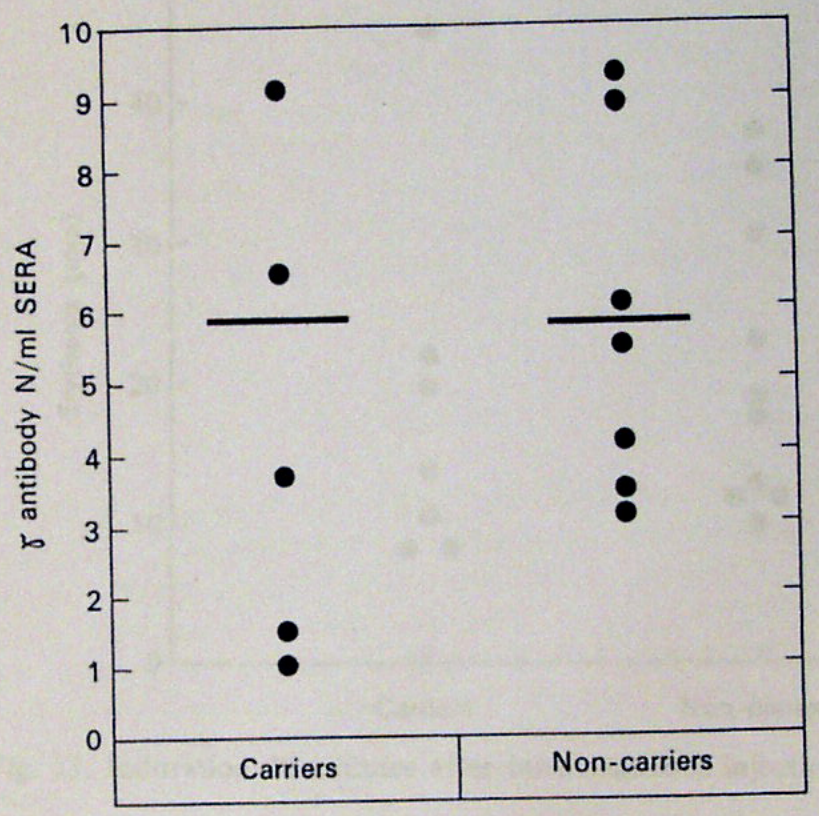

Fig. 11. Concentrations of teichoic acid antibodies in sera of carriers of staphylococci as compared to sera from non-carriers.

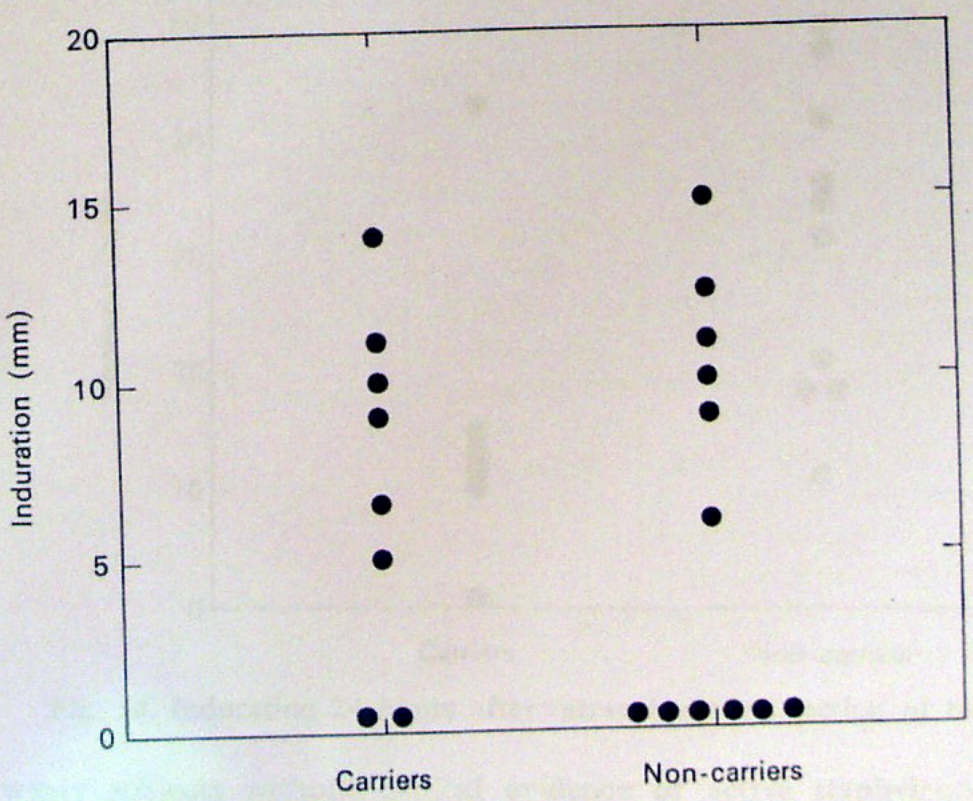

Fig. 12. Induration 24 hours after intracutaneous injection of protein A.

The concentrations of staphylococcal teichoic acid antibodies were determined by quantitative precipitin methods. Teichoic acid antibodies were present in all sera tested at similar concentrations in sera from nasal carriers of staphylococci as compared to sera from non-carriers (Fig. 11).

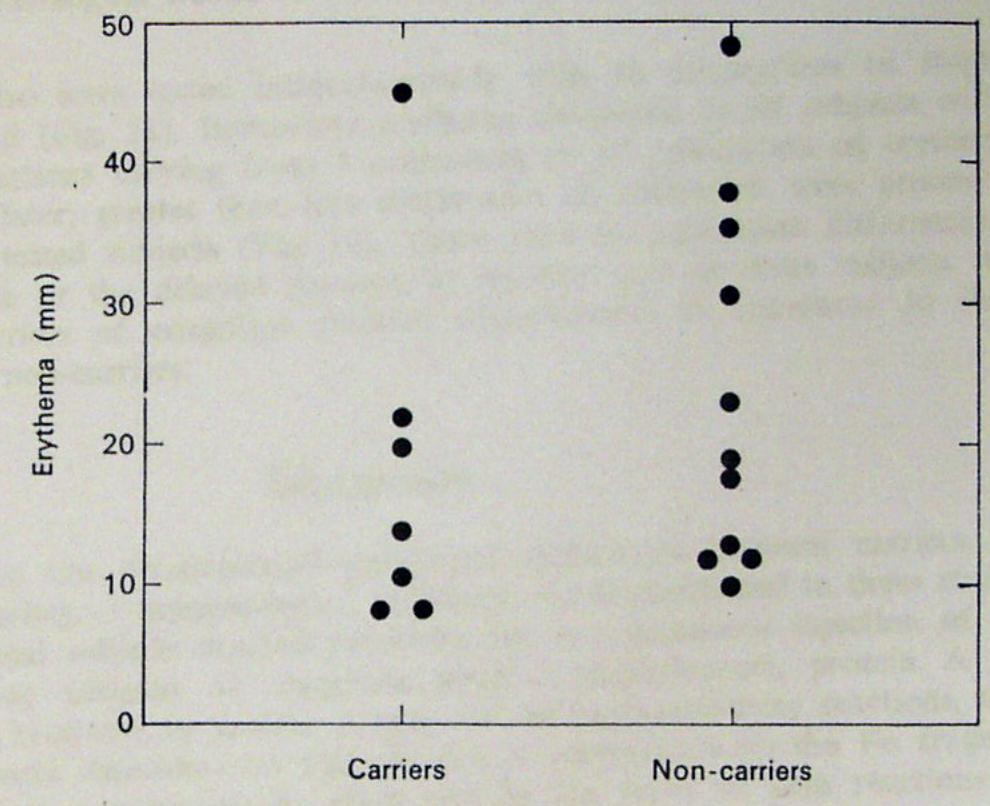

Fig. 13. Induration 30 minutes after intracutaneous injection of teichoic acid.

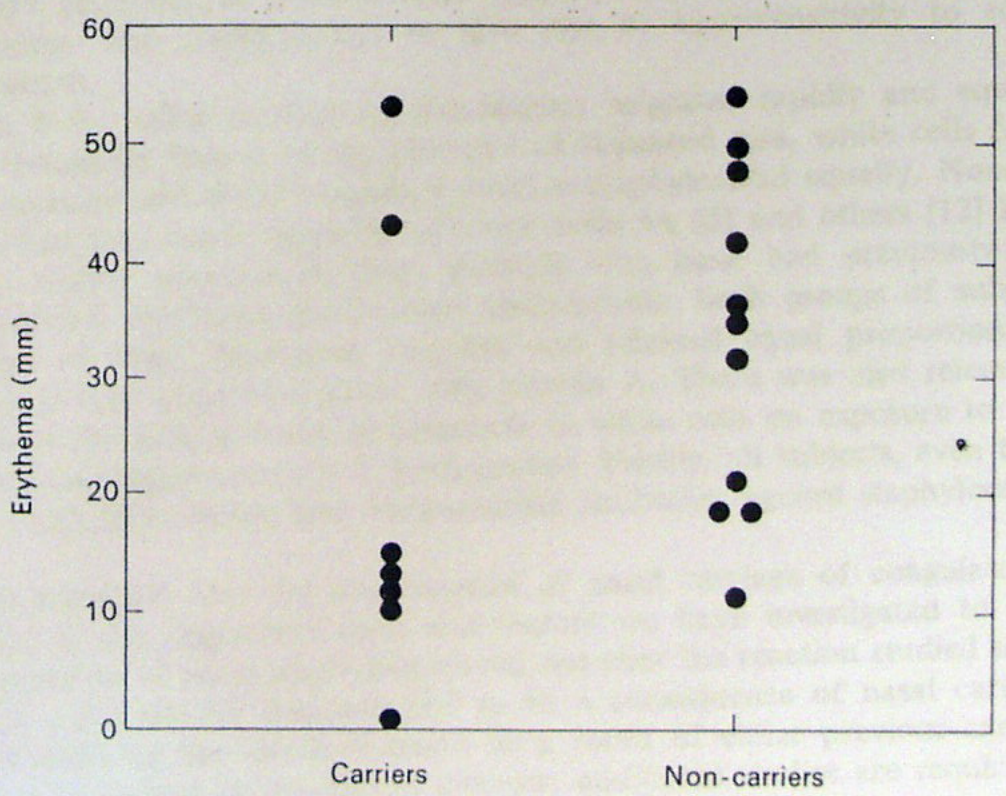

Fig. 14. Induration 24 hours after intracutaneous injection of teichoic acid.

Twenty subjects without clinical evidence of active staphylococcal diseases were tested intracutaneously with 200 micrograms of protein A (Fig. 12). Most of the subjects demonstrated greater than 5 millimeters of induration 24 hours after the injection. There were no significant differences in the reactivity of the 8 subjects who were consistant nasal carriers of coagulase positive staphylococci as contrasted to the 12 subjects who were non-carriers.

Eighteen subjects also were tested intracutaneously with 10 micrograms of staphylococcal teichoic acid (Fig. 13). Immediate erythema developed in all subjects within 30 minutes with reactions varying from 8 milimeters to 48 millimeters of erythema. Twenty-four hours later, greater than five millimeters of induration were present in all but one of the tested subjects (Fig. 14). There were no significant differences in either the immediate or the delayed reaction to teichoic acid in those subjects who were consistant carriers of coagulase positive staphylococci as compared to those subjects who were non-carriers.

Discussion

These data have not demonstrated significant differences between carriers and non-carriers in a variety of immunological reactions. As demonstrated in these studies, a majority of normal subjects reacted following the intracutaneous injection of two of the major group antigens of coagulase positive staphylococci, protein A and teichoic acids. The reactions to protein A may not be hypersensitivity reactions, since it has been previously demonstrated that protein A combines with the Fc fragment of IgG [4] and thus, non-specifically gives rise to the types of skin reactions demonstrated in those subjects skin tested with protein A.

However, the skin reactivity, to teichoic acids suggests that most adult subjects have had sufficient contact with staphylococci to give rise to hypersensitivity to some staphylococcal products.

The white cells from either carriers or non-carriers migrated rapidly and equally in response to a chemotaxic factor; in the presence of unheated sera, white cells from both groups phagocytized and killed coagulase positive staphylococci equally. None of the subjects tested had heat stable opsonins although both we [5] and others [12] have demonstrated heat stable opsonins in those patients who have had previously documented staphylococcal infections, particularly endocarditis. Both groups of subjects had similar content of WBC lysosomal enzymes and released equal proportions of histamine from white cells after incubation with protein A. There was also release of a large proportion of the total amount of histamine in white cells on exposure to very small quantities of homologous extracts in both groups. Finally, all subjects, even those without evidence of clinical disease, had demonstrable antibodies against staphylococcal teichoic acid.

All these studies suggest that the maintenance of nasal carriage of coagulase positive staphylococci is not dependent upon the factors we have investigated to date. In additions, the presence of nasal staphylococci did not alter the reaction studied so the observed reactions could not be demonstrated to be a consequence of nasal carriage. It is possible that some of the reactions could be a result of either previous carriage of staphylococci or of clinical or subclinical diseases; additional studies are required to differentiate these possibilities.

In future studies we plan to investigate the reactions between nasal secretions and staphylococci since this site should be the most frequent area of contact between staphylococci and host-defense mechanisms.

Acknowledgments

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