POSSIBLE ROLE OF OPSONINS IN ADSORPTION AND TRANSPORT OF BIOLOGICAL MEMBRANES AND VIRUSES BY STAPHYLOCOCCI

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Ingestion of bacteria by phagocytes depends on activation of certain mechanisms of opsonization [6], which is linked with the character of the structure and chemical composition of the surface structures of bacteria and with the presence of a wide range of different opsonins (C3b, IgG1, IgG3, and IgM antibody aggregates, α-globulins, possibly lysozyme, C-reactive protein, etc.). Data [11] showing that plasma fibronectin takes part in adhesion of staphylococci (but not of *Escherichia coli*) to neutrophils in man can serve as another example.

Two opinions are held on opsonization of bacteria: Receptors exist for opsonins, and opsonins are responsible for the hydrophobicity of bacteria, which can then make contact more easily with the plasma membrane of the phagocyte. The results of electron-microscopic investigations of streptococci [8] and staphylococci [2, 3], opsinized with blood serum, showed that an additional capsule-like layer 80-90 nm thick, consisting of immunoglobulins and other components of blood serum, appears on the cell wall of bacteria. Usually bacteria surrounded *in vivo* by a capsule-like cover, such as staphylococci, are regarded as capsule-forming bacteria [5]. However, investigation of bacteria treated with blood serum, and the study of experimental staphylococcal infection and also of pathological material from patients with staphylococcal infection has led to the conclusion that these formations (as regards staphylococci) are not a capsule, but a deposit of immunoglobulins and of other humoral factors (opsonins) [2, 3].

During suppurative inflammation interaction may take place between different microorganisms, including in the form of viral-bacterial associations [4], but the role of humoral factors in this process has not been adequately studied.

The aim of this investigation was to study the role of staphylococcal surface structures during contact with humoral and cellular factors of the host and the role of viruses in this process in a model of mixed viral-bacterial infection. Staphylococci were chosen as one representative of the microflora of the upper respiratory tract [1] and as possible participants in mixed infection in acute respiratory diseases.

EXPERIMENTAL METHOD

Capsule-free strain *Staphylococcus aureus* Wood-46, grown at 37°C for 16 h on nutrient agar, and influenza virus (Leningrad 385/80/H_3N2 strain), purified and concentrated by ultracentrifugation [7] in a sucrose density gradient, were used. The influenza virus concentrate contained 30 mg/ml of protein [9], 2.4 mg/ml of hemagglutinin [13], and 512,000 hemagglutination units; the extinction ratio was : E_{260} nm/E_{280} nm = 1.25. To study interaction between staphylococci and blood from a group O blood donor, 1 ml of a 10^9 bacterial suspension in isotonic sodium chloride solution. The mixture was kept for 10 min at a temperature of 37°C, after which it was divided into two parts. To stimulate mixed infection 0.05 ml of a 10% suspension of blood cells and influenza virus (ratio of the blood serum-blood cell system to the suspension of influenza virus 20:1) was added to one of them. The remainder of the incubation mixture (without virus) served as the control. An additional control also was set up at the same time, consisting of an incubation mixture of staphylococcus, virus, and isotonic sodium chloride solution. After a further 5 min of incubation at 37°C the material was processed for electron-microscopy by the method described previously [3]. The dehydrated material was

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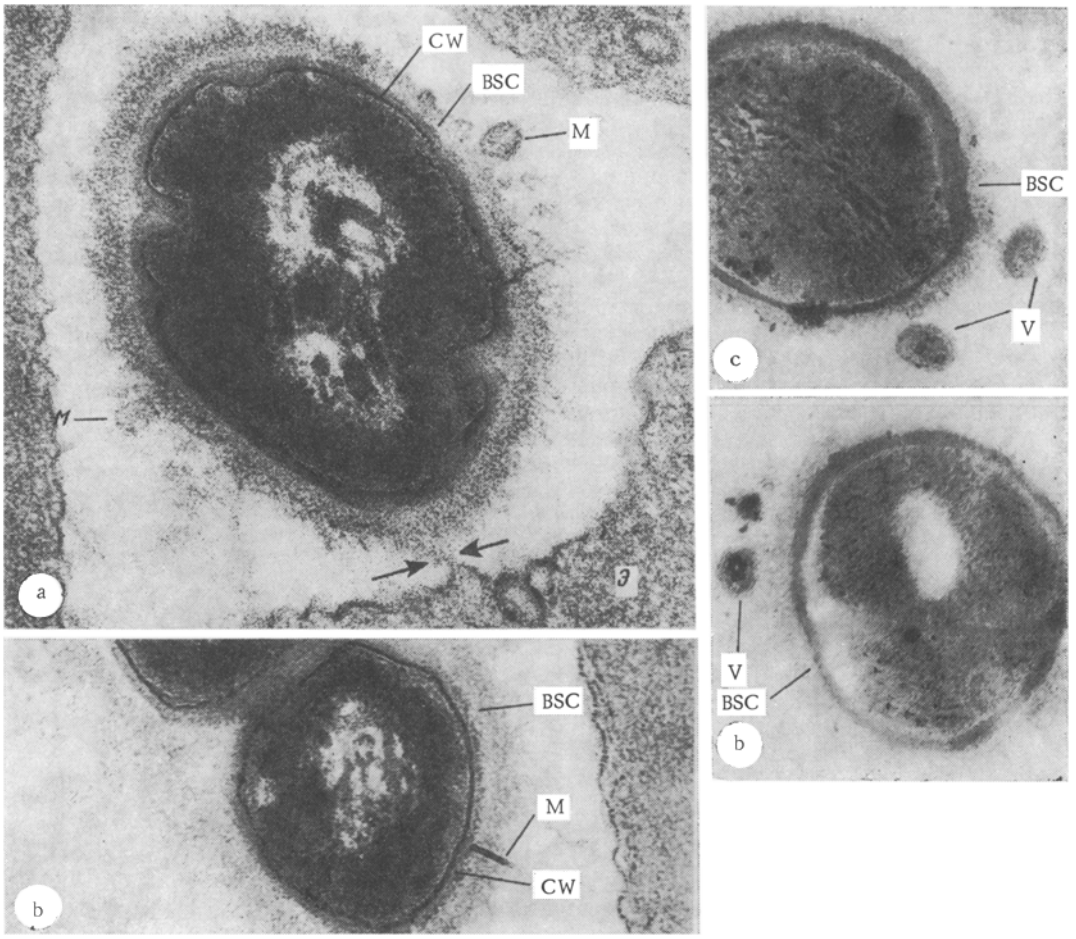

Fig. 1. Ultrathin sections through cells of *Staphylococcus aureus*, opsonized with blood serum and incubated with erythrocytes (a, b) and influenza virus particles (c, d). a) Immune adhesion (arrows) of staphylococcus to erythrocyte membrane and to concentric membranes through blood serum components adsorbed on the staphylococcal cell wall; b) adhesion of membrane fragment to staphylococcal cell wall by means of blood serum components; c, d) adsorption of influenza virus particles to staphylococcus by means of blood serum components. Here and in Fig. 2: M) membrane, E) erythrocyte, BSC) blood serum components, CW) cell wall, V) virus particles. Magnification: a, b) 72,000, c, d) 60,000.

embedded in Epon resins. Ultrathin sections were examined in the IET-7A electron microscope

EXPERIMENTAL RESULTS

Capsule-like structures, consisting of immunoglobulins and serum and blood components were detected on cells of the unencapsulated strain Wood-46 of staphylococcus, incubated with blood serum (Fig. 1a) and on bacteria treated with methicillin or killed with glutaraldehyde, then incubated with blood serum [3]. Bacteria surrounded with blood serum opsonins adhered to the erythrocytes (Fig. 1a) due to the effect of immune adhesion [2, 10, 12]. Possibly as a result of injury to the erythrocytes, concentric membrane structures, interacting with blood serum opsonins (Fig. 1a) located on the surface structures of the staphylococcus (immune adhesion), were formed. Fragments of membranes in contact with the cell wall of the staphylococcus and fixes to it by a layer of blood serum opsonins also were discovered (Fig. 1b).

The fact that interaction took place between opsonized staphylococci and membrane structures justified a further study of the ability of the virus particles to become fixed to these bacteria and transported as a result into the viral cell in the course of mixed viral-bacterial infection. Accordingly interaction between viruses and staphylococci was studied from the comparative aspect in systems of staphylococci—opsonins—virus (staphylococcus incubated with blood

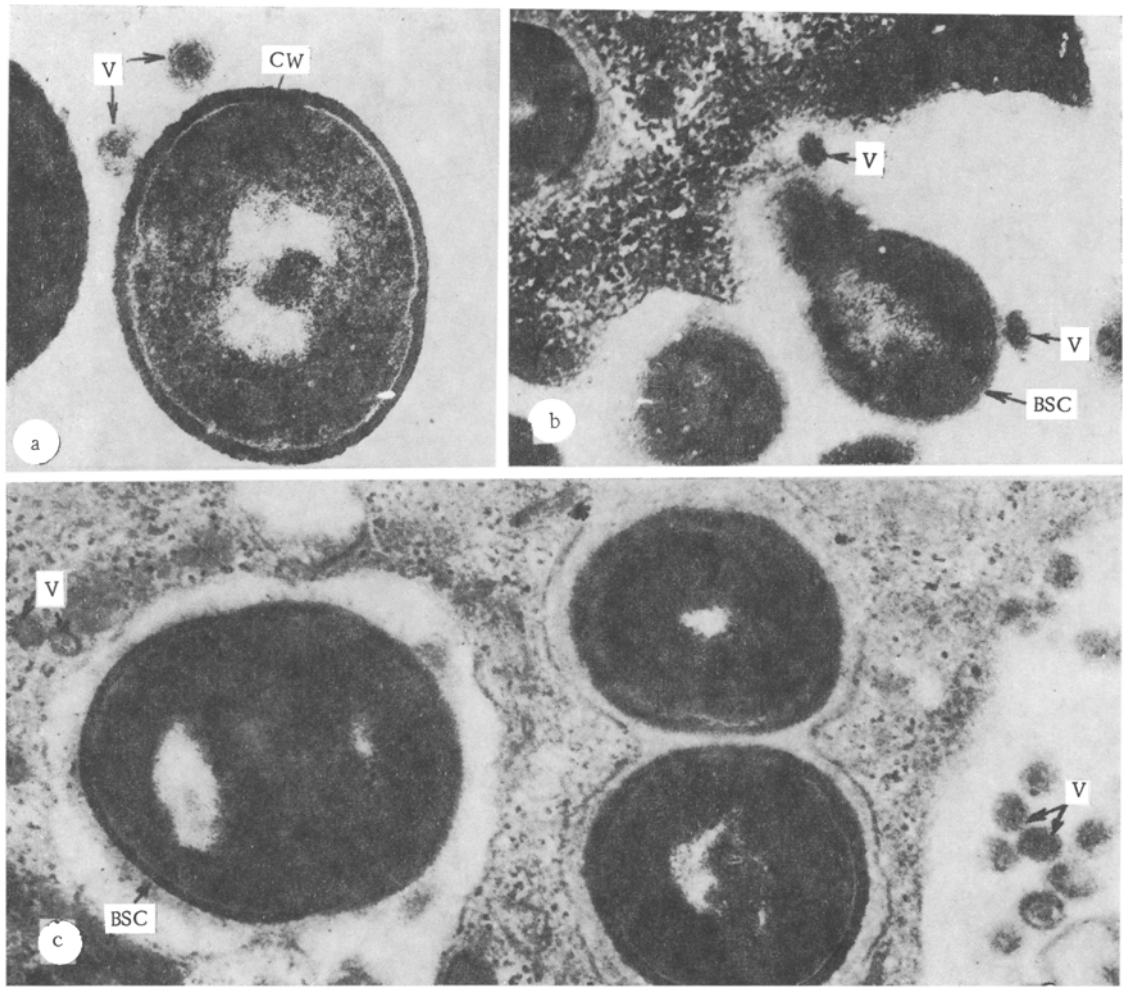

Fig. 2. Ultrathin sections through *Staph. aureus* cells incubated with influenza virus particles (a) and subjected to phagocytosis after opsonization of bacteria with blood serum (b, c). a) Adhesion of influenza virus particles to staphylococcal cell wall; b, c) adhesion of influenza virus particles to staphylococcus, opsonized with blood serum components, and phagocytosis of opsonized bacteria. Mangification: a, c) 60,000; b) 48,000.

serum and influenza virus) staphylococcus—influenza enza virus (control).

Intensive enveloping of the staphylococcus and less intensive envelopment of influenza virus particles by opsonins took place in the staphylococcus—opsonins—virus system. As a result of this process the staphylococci and virus particles were bound together by means of opsonins (Fig. 1c, d). In the control tests of the staphylococcus—virus system (without blood serum) no capsule—like structures were found. The virus particles in this case adhered directly to the staphylococcal cell wall (Fig. 2a). Staphylococci surrounded by blood serum opsonins, and carrying virus particles, adhered to the surface of polymorphonuclear leukocytes (Fig. 2b), and underwent capture, ingestion, and digestion (Fig, 2c). The opsonins were destroyed inside the phagosomes, Simultaneously with phagocytosis of the staphylococcus with adsorbed virus particles, phagocytosis also took place of virus particles not adsorbed on staphylococci, but located outside phagosomes containing bacteria.

Thus opsonized staphylococci can carry biological membranes and viruses on their surface, i.e., they can play the role of unique "carriers" of certain biological objects. This approach to bacteria, considered to be representative of the resident microflora of the upper respiratory tract, suggests that opsonization of staphylococci can take place on the surface of the mucous membranes of the upper respiratory tract, especially in the course of a pathological process accompanied by a higher level of humoral factors in the secretion from the mucous membranes. Opsonized staphylococci can interact with virus particles to form complexes, and

thus to participate in the development of mixed infection. The mechanism of mixed infections has been inadequately studied. It has been shown that during mixed infection the different agents can come into contact with each other; for example, virus particles may be adsorbed from the surface of other viruses or bacteria [4]. When they enter the body simultaneously, different processes may arise: besides interaction with other, the associated microorganisms may take part in complex interaction with the host's protective factors. The possibility cannot be ruled out that the staphylococcus–opsonins–virus complex which is formed may enter the environment, and facilitate the development of mixed infection. A similar effect may also be found with viremia. In this case viruses may perhaps leave the blood stream to enter a focus of inflammation caused by pyogenic bacteria, may be adsorbed on them, and may spread together with them in the evironment.

This demonstration that viruses can be adsorbed on bacteria, including opsonized bacteria, will enable the pathogenesis and epidemiology both of mixed infections, and of some respiratory and intestinal virus infections, during which frequent contact between viruses and the bacteria representative of the normal microflora of the human body is possible, to be studied more fully,

LITERATURE CITED

1. V. D. Belyakov, A. P. Kolesov, P. B. Ostroumov, and V. I. Nemchenko, Hospital Infection [in Russian], Leningrad (1976).
2. A. S. Bykov, E. P. Pashkov, I. S. Lazurenko, et al., Zh. Mikrobiol., No. 9, 102 (1981).
3. A. S. Bykov, I. S. Lazurenko, and A. S. Sleznev, Arkh. Patol., No. 9, 10 (1981).
4. A. F. Bykovskii, in: General and Special Virology [in Russian], Vol. 1, Moscow (1982), pp. 84-113.
5. B. V. Vtyurin, G. N. Dudnikova, and N. V. Chervonskaya, Arkh. Patol., No. 12, 32 (1981).
6. A. N. Mayanskii and D. N. Mayanskii, Essays on the Neutrophil and Macrophage [in Russian], Novosibirsk (1983).
7. N. N. Sokolov, T. Ya. Zhukova, and L. K. Malofeeva, in: Respiratory Virus Infections [in Russian], Leningrad (1973), pp. 74-77.
8. R. G. Emyanitoff, T. E. Ruckinsky, and D. C. Birdsell, Can. J. Microbiol., 22, 891 (1976).
9. R. Flores, Anal. Biochem., 88, 605 (1978).
10. R. A. Nelson, Science, 118, 733 (1953).
11. R. Proctor, E. Prendergast, and D. F. Mosher, Blood, 59, 681 (1982).
12. I. Roit, Essential Immunology, London (1974).
13. G. C. Shild, I. M. Wood, and R. W. Neuman, Bull. Wld. Hlth. Org., 52, 223 (1975).