THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 65:244–247, 1975

Vol. 65, No. 2
Printed in U.S.A.

THE LOCALIZATION AND DISTRIBUTION OF GRAM-POSITIVE COCCI IN NORMAL SKIN AND IN LESIONS OF ACNE VULGARIS

SADAO IMAMURA, M.D.

Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto, Japan

The localization of gram-positive cocci in the normal skin and in the lesions of acne vulgaris was investigated using fluorescein-labeled antiserum raised to gram-positive, coagulase-negative cocci. The cocci were found in 10 of 19 specimens from normal facial skin and in 3 of 11 specimens from the normal skin of the rest of the body. The bacteria were found mostly in the openings of follicles, but in 6 of 10 facial skin specimens, they were also present deeply in the lumina of the dilated sebaceous follicles near the sebaceous glands. Cocci were found in 5 of 6 noninflammatory acne comedones. In inflammatory acne they were demonstrated not only in the follicular canals but also sparsely in the infiltrate surrounding the follicles.

Corynebacterium acnes, coagulase-negative cocci and *Pityrosporum ovale* have been frequently cultured from follicular canals of normal skin and lesions of acne [1–4]. All three organisms are thought to play an important role in the pathogenesis of acne since in vitro experiments indicate that they produce free fatty acids from sebum triglycerides [5–7].

However, based on observations on free fatty acid levels in scalp lipids after the administration of selective antibiotics to patients with these organisms, Marples et al [8–10] showed that *C. acnes* was mainly responsible for lipolysis of triglycerides, although *P. ovale* had some activity when *C. acnes* density was low, and cocci played no part in lipase production in vivo.

Corynebacterium acnes is a gram-positive bacterium which is difficult to differentiate from gram-positive cocci in tissue sections. In a previous report, we demonstrated the localization and distribution of *C. acnes* in normal skin and acne lesions using an immunofluorescent technique [11]. The present investigation focused on the demonstration of cocci in the tissues of normal skin and acne lesions. Fluorescein isothiocyanate-labeled antiserum to gram-positive, coagulase-negative cocci was used for the studies.

MATERIALS AND METHODS

A suspension of formalin-killed bacteria was prepared from gram-positive, coagulase-negative cocci which were isolated from an acne comedo. Albino rabbits weighing 2.0 to 2.5 kg were given an intramuscular injection of a mixture of 10 ml of the bacterial suspension at a concentration of 100 mg per ml and an equal volume of complete Freund's adjuvant. Four weeks later, they were given three courses of intravenous injections consisting of inoculation on 3 successive days and a rest of 4 days. The first injection consisted of 0.5 ml of the bacterial suspension at a concentration of 1 mg per ml, and this was increased 0.5 ml each day until 4.5 ml was injected. The rabbits were bled 5 days after the last injection. Agglutination titers in sera were 1:5120.

The crude globulin fraction of the antiserum was conjugated with fluorescein isothiocyanate and the conjugate was purified through a DEAE-cellulose column as previously described [11]. Preparation of smears and sections, and staining with the conjugate were also performed as previously described [11]. Examination of specimens was done through a Zeiss fluorescence microscope, equipped with Osram HBO 200 maximum pressure mercury lamp. Photomicrographs were recorded on 35 mm Kodak Tri-X Pan film.

The specificity of the staining reaction was confirmed by the absence of staining reaction of the adjacent sections after absorption of the conjugate with coagulase-negative cocci.

RESULTS

Staining of Bacterial Smears

The specificity of the conjugate was initially evaluated using smears of homologous and heterologous bacteria. As shown in the Table, the conjugate reacted intensely to gram-positive cocci (Fig. 1), but did not react to *C. acnes*, *P. ovale*, or *E. coli*.

There was not much difference in the reactivity of the conjugate to various Baird-Parker types of gram-positive cocci. In order to eliminate the reactivity of the conjugate to gram-positive, coagulase-positive cocci (*Staphylococcus aureus*), absorption of the conjugate with *S. aureus* was attempted. Although the absorbed conjugate did not react to *S. aureus*, the reactivity to other types of the cocci also almost disappeared. Accordingly, the present experiments were carried out using unabsorbed conjugate, and the reaction was considered to indicate the localization of gram-posi-

Manuscript received November 27, 1972; in revised form February 24, 1975; accepted for publication March 12, 1975.

Supported in part by Basic Research Fund of the Japanese Dermatological Association, Inc.

Reprint requests to: Dr. S. Imamura, Department of Dermatology, Faculty of Medicine, Kyoto University, Shogo-in, Sakyo-ku, Kyoto 606, Japan.

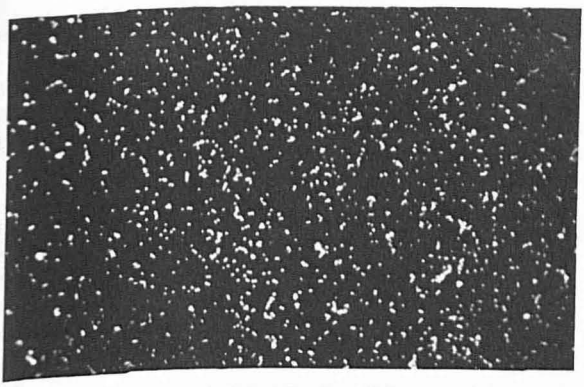

FIG. 1. Smear of Baird-Parker SII reacted intensely with fluorescein-labeled, gram-positive, coagulase-negative antiserum (× 320).

TABLE. *Innunofluorescent staining of bacteria with fluorescein-labeled, gram-positive, coagulase-negative cocci antiserum*

Bacteria		Immuno-fluorescence
Corynebacterium acnes		–
Pityrosporum ovale		–
Escherichia coli		–
Staphylococci and micrococci		
Baird-Parker type	SI (*S. aureus*)	++
	SII	+++
	SIII	Not done
	SIV	+++
	SV	+++
	SVI	+++
	M1	++ ~ +++
	M2	+++
	M3	++ ~ +++
	M4	++
	M5	Not done
	M6	Not done
	M7	++

tive cocci (staphylococci and micrococci) in general.

Localization of Cocci in Normal Skin

Nineteen biopsy specimens of normal skin were taken from the face and 11 from other skin, including 3 from the abdomen, 3 from the arm, 2 from the chest, 2 from the back, and 1 from the buttocks. Although many kinds of autofluorescence, (light-blue fluorescence of the horny layer, horny mass, hair shafts within the follicles, and elastic fibers; dark-blue fluorescence from collagen fibers) were visible in the sections, they were readily distinguishable from the apple-green color of the specific fluorescence of the cocci.

In facial skin, the cocci were demonstrated in the horny layer and in the follicular canals. However, the cocci in the horny layer were not taken into consideration in the present study, because the most superficial portion of the horny layer may have been removed during processing of the sections. Cocci were found in the follicular canals in 10 of 19 specimens, and they were scattered mostly in follicular orifices. In 6 to 10 specimens, however, cocci were also detected in the lumina of the dilated sebaceous follicles deeply near the sebaceous glands, and, in addition, small quantities of amorphous bacterial antigen which was demonstrable as diffuse apple-green fluorescent material was seen (Fig. 2). The cocci and their antigens in the follicular lumina were not as prominent as *C. acnes* which often formed large masses and filled the entire lumen of the follicles [11]. Cocci and their antigen were never observed within the sebaceous gland itself.

In the skin other than from the face, cocci were found only in 3 or 11 specimens. The bacteria were limited to the openings of the follicular canals, and no cocci were observed below the infundibulum (Fig. 3).

Localization of Cocci in Acne Lesions

Six biopsy specimens of noninflammatory acne comedones and 13 of inflammatory acne lesions were taken from the face.

Noninflammatory acne comedones. The cocci and their antigens were demonstrated in 5 to 6 specimens of comedones. They were found between

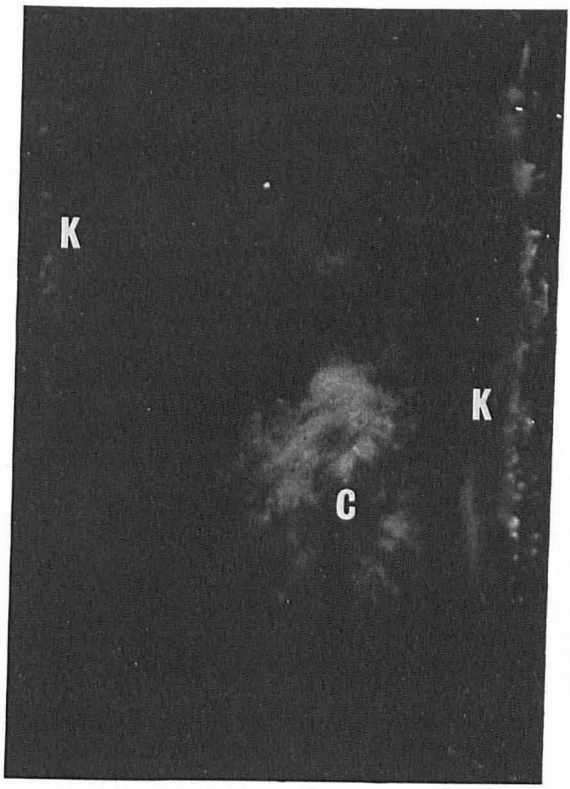

FIG. 2. Sebaceous follicle of the face. Cocci and their antigens (amorphous material) (*C*) are seen in the lumen. Autofluorescence of keratohyaline granules (*K*) of the follicular epithelium is shown near margins (× 680).

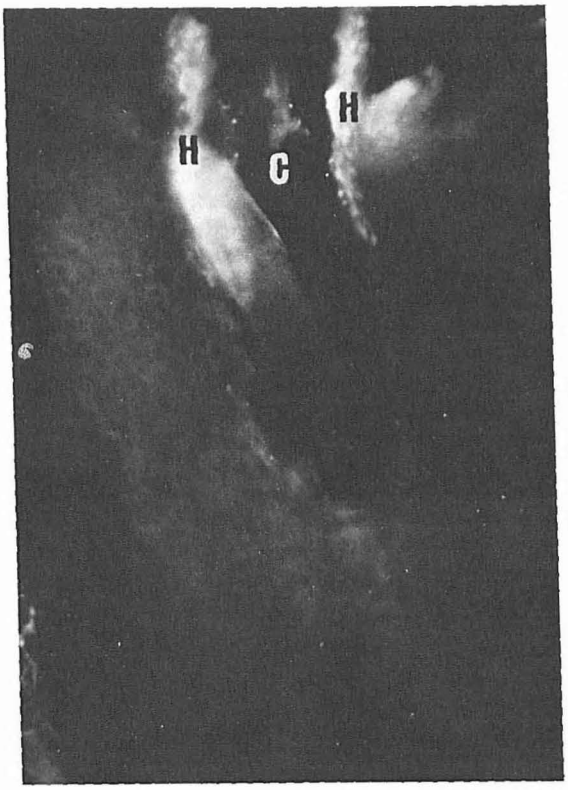

FIG. 3. Follicular canal of the abdominal skin. Cocci (*C*) are scattered in the opening of the canal. Horny masses (*H*) show autofluorescence (× 280).

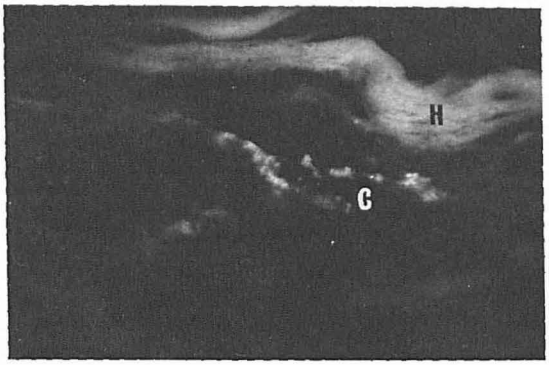

FIG. 4. Noninflammatory acne comedo. Cocci and their antigens (*C*) are seen between the horny masses (*H*) which show autofluorescence (× 475).

the horny masses of the comedones (Fig. 4). Individual bacteria were often easily identified in the bacterial masses, in contrast to *C. acnes* which formed compact masses in which individual bacteria could not be identified [11].

Inflammatory acne lesions. The inflammatory acne lesions examined consisted of 6 papules and 7 pustules. Cocci and their antigens were demonstrated in 3 of 6 papules and 4 of 7 pustules. The bacteria were found not only in the follicular canals but also in the inflammatory infiltrate around the follicles. In the follicular canals, the bacteria were seen between the horny masses just as in noninflammatory comedones. On the other hand, the quantity of cocci in the inflammatory infiltrate was surprisingly small. Only a few bacteria were seen outside of the inflammatory cells.

DISCUSSION

Although many types of gram-positive cocci are isolated from the normal skin and acne lesions, it has been reported that Baird-Parker Type SII was predominant both in normal face and acne lesions [12]. In order to demonstrate the precise localization of a given type of cocci by means of an immunofluorescent technique, a strictly specific antiserum against the cocci is required. The coagulase-negative cocci antiserum prepared for the present study revealed strong cross-reactivity among various Baird-Parker types of cocci. Even the absorption procedure with *S. aureus* failed to produce the specifically reactive conjugate to coagulase-negative cocci. This may be due to common antigens contained in the gram-positive cocci [13].

In normal skin other than from the face, cocci were found only in the openings of the follicles, and no cocci were seen below the infundibulum. This agrees with the findings of other investigators [14, 15]. In contrast, in the dilated sebaceous follicles of the face, cocci were also found deeply below the infundibulum of the follicular canals near the sebaceous glands. This finding is qualitatively similar to that of *C. acnes*, although the latter bacteria were much more densely found in large masses [11].

Both in the lesions of noninflammatory acne comedones and in the lesions of inflammatory acne, the localization of the cocci was similar to that of *C. acnes* [11]. However, cocci were present in much lower concentration. This differs from the findings of Izumi, Marples, and Kligman [3] and Marples and Izumi [4], who cultured roughly equal numbers of *C. acnes* and aerobic cocci from lesions of pustular acne, although *C. acnes* outnumbered cocci in acne comedones.

REFERENCES

1. Shehadeh NH, Kligman AM: The bacteriology of acne. Arch Dermatol 88:829–831, 1963
2. Ganor S, Sacks TG: A comparison of the flora of the comedones of acne vulgaris and comedones in elderly people. Dermatologica 138:1–9, 1969
3. Izumi AK, Marples RR, Kligman AM: Bacteriology of acne comedones. Arch Dermatol 102:397–399, 1970
4. Marples RR, Izumi AK: Bacteriology of pustular acne. J Invest Dermatol 54:252–255, 1970
5. Freinkel RK: The origin of free fatty acids in sebum. I. Role of coagulase-negative staphylococci. J Invest Dermatol 50:186–188, 1968
6. Reisner RM, Silver DZ, Puhvel M, Sternberg TH: Lipolytic activity of *Corynebacterium acnes*. J Invest Dermatol 51:190–196, 1968
7. Reisner RM, Puhvel M: Lipolytic activity of *Staphylococcus albus*. J Invest Dermatol 53:1–7, 1969
8. Marples RR, Kligman AM, Lantis LR, Downing DT: The role of the aerobic microflora in the genesis of

free fatty acids in human surface lipids. J Invest Dermatol 55:173–178, 1970
9. Marples RR, Downing DT, Kligman AM: Control of free fatty acids in human surface lipids by *Corynebacterium acnes*. J Invest Dermatol 56:127–131, 1971
10. Marples RR, Downing DT, Kligman AM: Influence of Pityrosporum species in the generation of free fatty acids in human surface lipids. J Invest Dermatol 58:155–159, 1972
11. Imamura S, Pochi PE, Strauss JS, McCabe WR: The localization and distribution of *Corynebacterium acnes* and its antigens in normal skin and in lesions of acne vulgaris. J Invest Dermatol 53:143–150, 1969
12. Marples RR: The microflora of the face and acne lesions. J Invest Dermatol 62:326–331, 1974
13. Julianelle LA, Wieghard CW: Immunological specificity of staphylococci. III. Interrelationship of cell constituents. J Exp Med 62:11–21, 1935
14. Marples MJ: The Ecology of the Human Skin. Springfield, Ill, Thomas, 1965
15. Kligman AM: The bacteriology of normal skin, Skin Bacteria and Their Role in Infection. Edited by H Maibach, G Hildick-Smith New York, McGraw-Hill, 1965.