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SCANNING ELECTRON MICROSCOPIC STUDY OF THE COMPLEMENT RECEPTOR SITE ON HUMAN DIPLOID FIBROBLASTS.

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Abstract

Sheep red cells, treated with 19S antibody and mouse serum as the source of complement (EAC), interact with human diploid fibroblasts and the immune adherence occurs owing to the existence of the complement receptor site on the cell membrane of human fibroblasts. Examined under a scanning electron microscope, EAC attach only to the microvilli of fibroblasts, and at that time many microvilli with free end can be observed on the cell membrane of fibroblasts. On the contrary the cell surface of normal fibroblasts, which have not incubated with EAC, seems to be relative smooth and microvilli are not so prominent.

It might be speculated that fibroblasts posess the complement receptor site on the top of microvilli, and the microvilli become prominent when the cell membrane is activated with some stimuli.

It has been reported that several types of cells posess the mechanisms of some recognition or specific receptors on the cell membrane (1-5). Among these receptors, the one specific for complement molecules has been found on erythrocytes (6), macrophages (1-3), B-lymphocytes (1), granulocytes (4), monocytes (4) and platelets (4). Recently we have found the receptor site for the complement on the cell surface of human fibroblasts (7).

It is the purpose of this paper to describe the features of the complement receptor site on human diploid fibroblasts under a scanning electron microscope after several intervals following to the treatment of human fibroblasts with sheep red cells treated with 19S antibody and mouse complement (EAC).

MATERIALS AND METHODS

Sheep erythrocyte (*E*) Sheep red cells commercially obtained and stored in Alsever's solution were washed three times in phosphate buffered

saline (PBS) at pH 7.2 and adjusted to a concentration of 1×10^9 cells/ml in PBS before use.

Treatment of E with 19S rabbit antibody and mouse complement (C) As described in the previous paper (7), rabbit antiserum to erythrocytes stroma was obtained, and a 19S fraction of the antiserum was prepared by applying the serum to a Sephadex G-200 column which had been equilibrated with 0.15M Nacl-0.02M tris-Hcl buffer (pH 8.0).

Aggulutination tests were performed with 1% sheep red cells using the 19S fraction of antibody. Equal volume of 1×10^9 cells/ml suspension of washed E and 19S fraction of the antibody, at a concentration at which hemagglutination does not occur, were incubated at 30°C for 30 minutes.

The sensitized cells (EA) were washed three times and incubated with fresh AKR mouse serum at 37°C for 15 minutes. Details were described in the previous report. The cells (EAC) were washed and adjusted to 1×10^8 cell/ml in PBS.

Human diploid fibroblasts Strain WI-38 cells were commercially obtained from Flow Laboratory (USA) and cultured on cover slips with minimum essential medium (MEM) and 10% fetal calf serum in Leighton tubes at 37°C for 2 days.

Incubation of human diploid fibroblasts with EAC Cover slips with WI-38 cells were washed three times in PBS and incubated aseptically in glass tubes with 0.8 ml of EAC suspension, adjusted to 1×10^8 red cells/ml in PBS. After the incubation at 37°C for 1 hr and subsequently for 1 hr at room temparature, they were washed three times in PBS.

Scanning electron microscopy The specimens mentioned above and some cover slips with WI-38 cells, which were not incubated with EAC, were fixed with 1% glutaraldehyde (pH 7.3 320 mosmol), rinsed in PBS (pH 7.2). They were dehydrated in a grade series of alcohol, a grade series of amyl acetate/absolute alcohol and absolute amyl acetate. Then the specimens were dried in a Hitachi critical point apparatus (HCP-1) and coated with a thin layer of carbon and gold rotatory stage (Hitachi vacuum apparatus, HUS-4GB). They were examined using a Hitachi SSM-2 scanning electron microscope at a accelerating voltage of 20kV.

RESULTS

When we examined the specimens under a scanning electron microscope, the surface of normal fibroblasts, which were not incubated with EAC, seems to be relative smooth, and cytoplasmic processes or micro-

villi are not so obvious on the cell membrane (Fig. 1, 2). As seen in Figs. 1, 2 nuclear area and some nucleolei under the cell membrane can be observed, and fibroblasts attach to the glass with some longer cytoplasmic processes. Some end pads of microvilli can be observed on the cell surface of untreated fibroblasts, as seen in Fig. 2, but not so frequent as on the fibroblasts, which were incubated and reacted with EAC. On the contrary the fibroblasts, which were incubated with EAC, had formed rosette with EAC because of the immune adherence, so they were considered to posess the receptor sites for complement(s) on the membrane (7). With high magnifications the EAC adhered to WI-38 fibroblasts have not attached directly to the cell membrane of fibroblasts, but to the end of many microvilli of fibroblasts (Fig. 3, 4). Moreover there can be observed abundant microvilli with the free end, which seems to have met no chance to react with EAC. The average breadth of microvilli is 80 nm and the length is generally up to 1800 nm. It is of much interest that great differences can be seen in the cell surface of fibroblasts between in the control group and in the specimens after the reaction with EAC.

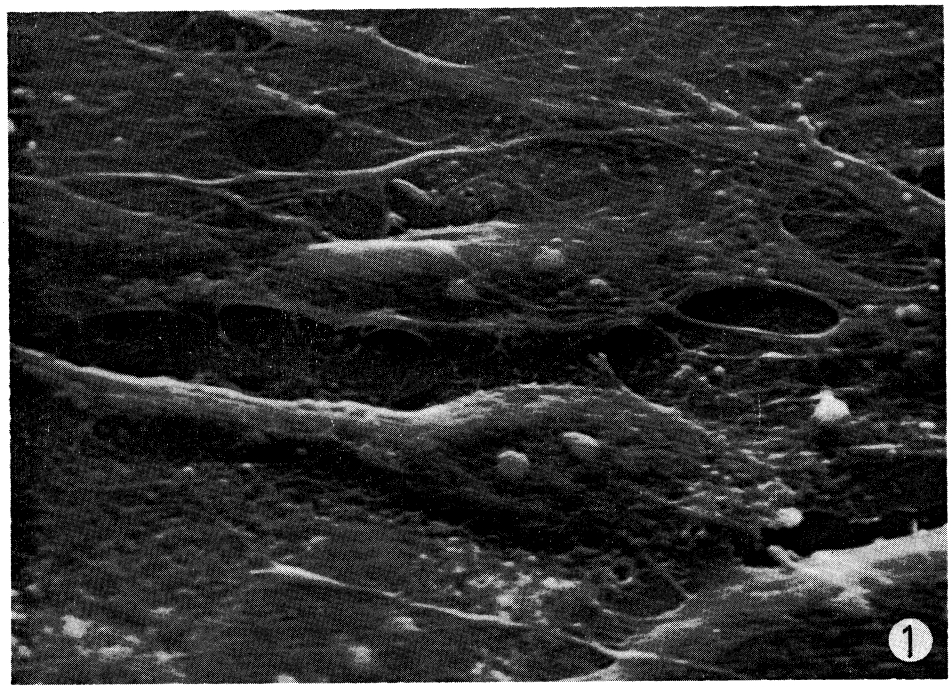

Fig. 1. Human diploid fibroblasts untreated with EAC. X 2800

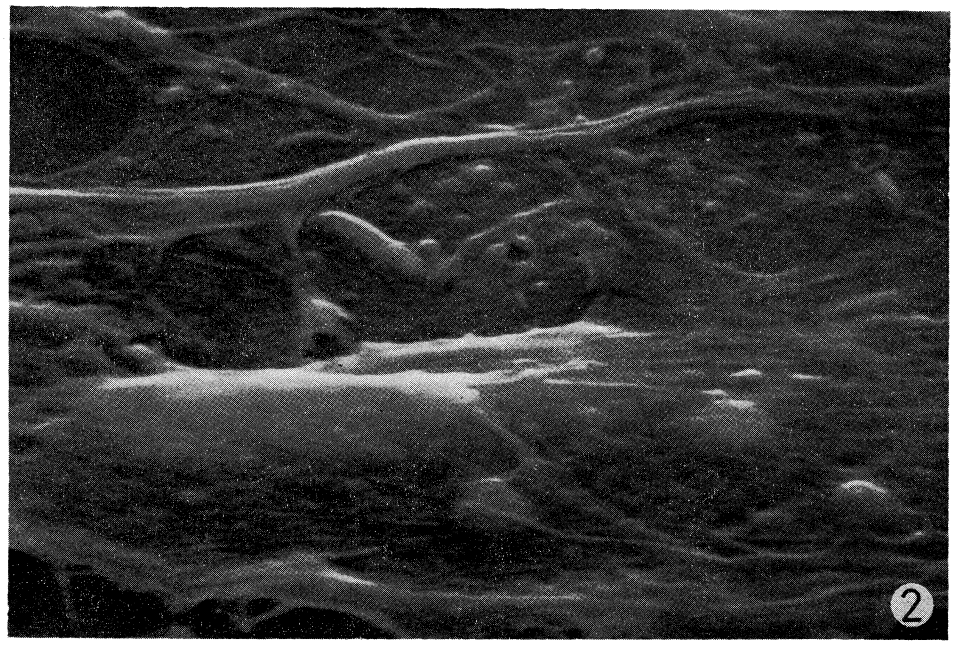

Fig. 2. The same fibroblasts as in Fig. 1, examined with high magnification. The cell surface is smooth and cytoplasmic processes are rare X 7600

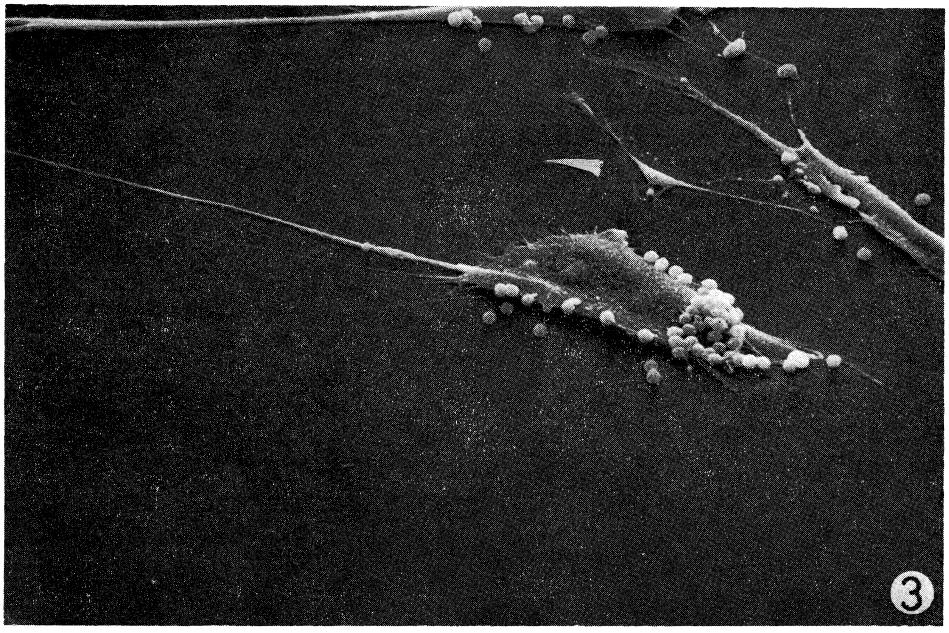

Fig. 3. Human diploid fibroblasts treated with EAC. Immune adherence can be observed, and the surface of fibroblasts seems not to be smooth, but to be rough because of many cytoplasmic processes. X 680

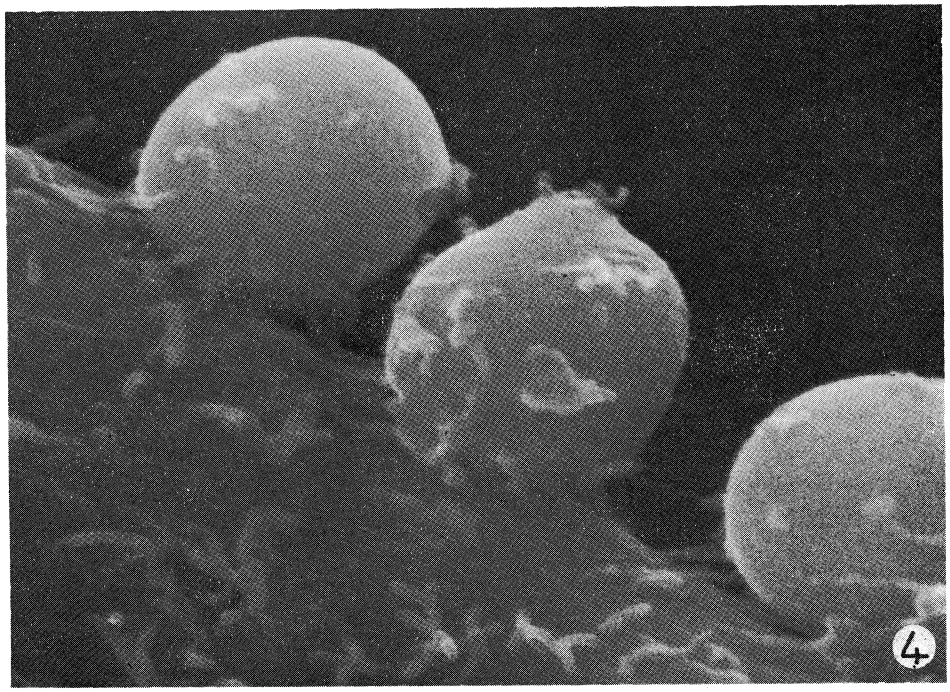

Fig. 4. EAC adhered to a fibroblast. They seem to attach to the end of microvilli and there can be observed a space between EAC and the cell surface of fibroblasts. X 15000

DISCUSSION

In the previous paper we have reported the existence of complement receptor site on the cell membrane of human normal fibroblasts (7). In this paper we have studied the ultrastructural features of human intact fibroblasts surface and the receptor site for the complement on the membrane of fibroblasts.

When examined under a scanning electron microscope, the EAC adhered to WI-38 fibroblasts do not attach directly to the cell membrane of fibroblasts, but to the end of microvilli. It may be speculated that fibroblasts posess the receptor site(s) for complement on the top of microvilli, at least when the both cells attach together. Since we cannot detect so many microvilli on the cell surface of intact fibroblasts, we may suspect that microvilli on the fibroblasts become prominent when the cell membrane is activated with some stimuli.

It has been known that fibroblasts play an important role in the inflammation, especially in the chronic inflammation. It is suspected that the complement receptor on the cell membrane of human fibroblasts

might have vital importance to the organism, but the details remain to be unkown.

In this study EAC appear to be a sphere with some short cytoplasmic spicules, and this form is "sphero-echinocytes" termed by Brecher et al. (8), owing to the treatment in saline. On the other hand, some authors reported that erythrocytes sphered following to the attachment to the macrophages (9) or monocytes (10). Details are to be clarified.

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