NOTE

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STREPTOCOCCAL PREPARATION AS AN ACTIVATOR OF HOST-MEDIATED IMMUNE RESPONSE: CELLULAR IMMUNITY AND ALTERNATE PATHWAY OF COMPLEMENT

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Streptococcal preparation, OK-432, was examined for its ability to initiate a host-mediated immune response. Aged individuals with negative skin response to phytohemagglutinin (PHA), as well as reduced in vitro blastoid transformation of lymphocytes, were treated with OK-432, and the response to PHA appeared in two out of three cases. The preparation was also demonstrated to convert C3 proactivator of complement when incubated with fresh human serum, as tested by immunoelectrophoresis, indicating the possibility that OK-432 might activate the alternate pathway of complement.

The streptococcal preparation, OK-432, has been developed as an anticancer agent by Okamoto et al.8,9) based on the evidence that tumor-bearing individuals are often relieved from the disease when suffering from streptococcal infection like erysipelas. The mode of action of this preparation has been explained partly by the direct action on malignant tumor cells and mostly by initiation of host-mediated immune responses,11) but no precise mechanism has yet been clearly established.

Previously, we have reported6) the phytohemagglutinin (PHA) skin test and concluded that this test provides a valuable indicator of immune deficiency in aged individuals. The present paper reports the effect of OK-432 on the cellular response to PHA in immune-deficient aged subjects, and also the humoral response of complement system, probably of alternate pathway.

Materials and Methods

Subjects Hospitalized aged individuals found to show negative PHA skin test and/or decreased PHA blastoid transformation of lymphocytes reported in the previous paper6) were examined.

PHA Skin Test Bacto-phytohemagglutinin P (Difco) dissolved in saline at a concentration of 100 μg/ml was intradermally injected in the forearm in 0.1 ml volume.

Lymphocyte Culture Blastoid transformation of lymphocytes in vitro by PHA was made by the modification of Caron et al.4) Normal range of tritiated thymidine (3H-TdR) uptake in our laboratory is from 2,000 to 5,000 cpm/10⁶ cells.

Immunoelectrophoresis Electrophoresis was performed in the presence of EDTA in Agarose. Antiserum to C3-proactivator and C3 component of the complement (β1C/βA) was commercially obtained from Behring Werke, Germany.

Streptococcal Preparation OK-432 (Picibanil) was donated by Chugai Pharm. Co., Tokyo. The dose is expressed as KE (Klinische Einheit), and 1 KE corresponds to 0.1 mg of the streptococcus in dried weight.

Results

Effect of OK-432 on Individuals with Negative PHA Skin Test Four aged individuals, showing negative PHA skin test with markedly reduced in vitro PHA blastoid transformation, were treated with daily in-
tramuscular injection of 1 KE of OK-432.

During this investigation, one unfortunately died of cerebral hemorrhage without being examined for PHA skin test. After 40 days of the treatment, two showed significant increase in PHA skin test, while one did not respond to the therapy. The latter died of upper respiratory infection before the in vitro examination of blastoid transformation, while the first two showed marked elevation of blastoid transformation as shown in Table I.

**Effect of OK-432 on Alternate Pathway of the Complement**

Aliquots of 1 ml fresh normal human serum were incubated with an equal volume of either saline, 0.1 or 0.5 KE of OK-432, or EDTA-saline solution, at 37 °C for 15 min, and the samples were examined by immunoelectrophoresis against anti-C3 proactivator and anti-C3.

As shown in Fig. 1, fresh serum incubated with OK-432 revealed partial electrophoretic change of C3-proactivator, from glycine-rich β-glycoprotein to glycine-rich γ-glycoprotein, as well as C3, from β1C to β1A, suggesting that alternate pathway of the complement was initiated by OK-432. Only a trace of degradation was observed in the sample incubated with saline, and none with EDTA-saline.

Sera from these patients were examined for the electrophoretic change of β-glycoprotein and C3, before and after the therapy, but no transformation of β- to γ-glycoprotein was demonstrated, and even the response to PHA became positive.

**Discussion**

Since the discovery of the ability of phytohemagglutinin (PHA) to stimulate human lymphocytes to undergo blastoid transformation, several investigations on immune-deficient subjects and tumor-bearing individuals have been reported, and the reaction of PHA in vitro has been recognized as a valuable method for examining the cellular immune system in man. Because of complex and laborious procedure of the in vitro examination, Blaese et al. developed a procedure.
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of intradermal administration of PHA and concluded that the PHA skin test provides a useful information in the evaluation of cellular immune deficiency in infants and children, while it does not indicate the presence of clinical anergy in adults since the correlation between in vivo and in vitro responses to PHA was difficult to be demonstrated in them. However, Kondo et al. examined PHA skin test in hospitalized aged individuals and reported that the method showed good correlation with the in vitro test; 7 out of 59 aged people, mainly suffering from sclerotic vascular diseases, were found to show negative skin response to either PHA or PPD (tuberculin), and six of them showed markedly decreased and one moderately low blastoid transformation of lymphocytes.

Therapeutic effect of OK-432 against malignant neoplasm has been reported by many investigators and the mode of action of this preparation is explained as mainly due to the initiation of host-mediated immune response. However, no precise explanation has been made on how the immune response can be initiated. Since the host-mediated immune responses in patients with malignant tumor are often modified by many factors, and the immune reactivity can change with the growth of tumor, it is difficult to demonstrate the therapeutic effect of immune stimulants in these patients. Therefore, immune-deficient aged subjects, already reported in our previous paper, were further investigated for demonstrating the change of immune responses when treated with OK-432, the streptococcal preparation. The fact that two patients, one with sclerotic heart disease and the other with rheumatoid arthritis, both showed negative skin test as well as markedly reduced in vitro reaction against PHA, appeared to show significantly accelerated skin response to PHA and in vitro blastoid transformation of lymphocytes, indicating the cellular immune system was greatly activated by the use of OK-432.

It is also well known that the complement system plays an important rôle in the humoral immune response. Despite the increasing knowledge on the complement, the rôle of a complement in the defence mechanism against neoplasm in clinical field has not been clearly demonstrated, since the change of complement level in the clinical course of patients is not sufficient to discuss immunological abnormalities. The complement system, usually explained as being activated by the initiation of antigen-antibody reaction and resulting in tissue damage or cell-lysis after a sequential reaction of the nine components, has been referred to as the classical pathway. However, it has become apparent in recent years that components from C3 and later can be activated by another route, called the alternate pathway. Bacterial lipopolysaccharide is one of the factors participating in the initiation of this pathway. By the activation of this alternate pathway, C3 proactivator, having an electrophoretic mobility in the y-globulin region and designated as glycine-rich y-glycoprotein, is converted into a major fragment of y-globulin mobility, i.e., glycine-rich y-glycoprotein. Therefore, the conversion of y- to y-glycoprotein is an indirect proof of the activation of the alternate pathway. The fact that OK-432 was effective in transforming y-glycoprotein in fresh human serum to y-glycoprotein suggests the initiation of this pathway by this streptococcal preparation, OK-432.

Although conversion of y- to y-glycoprotein in vivo could not be demonstrated in the serum from the patients treated with OK-432 and appearing to show positive PHA skin test, this seems natural, since the cleaved fragment can probably be cleared from the circulating blood immediately.

It is still obscure what the effective factor present in OK-432 is to initiate either the cellular immunity or the alternate pathway of the complement. Bacterial lipopolysaccharide might possibly be responsible for the
latter. However, it must be concluded that the streptococcal preparation, OK-432, might have a valuable immunotherapeutic effect against neoplasms through the activation of cellular immune response and complement system.

Of course, it is doubtful whether it is reasonable to compare the immune deficiency in tumor-bearing individuals with that in aged subjects, but the data reported here might be of help in investigating the mode of action of OK-432 as an immunotherapeutic agent against malignant tumors.

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REFERENCES