THE USE OF β-CYCLODEXTRIN IN LYMPHOCYTE CULTURE. INDUCTION OF THE PRIMARY ANTIBODY RESPONSE IN VITRO IN CALF SERUM- OR NEWBORN BOVINE SERUM-CONTAINING CULTURE MEDIUM

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In order to induce the primary antibody response to sheep erythrocytes in vitro in murine lymphocytes, the cells are usually cultured in RPMI-1640 medium containing 10% fetal calf serum (FCS). When FCS was replaced by calf serum (CS) or newborn bovine serum (NBS), the antibody response was poor compared with that obtained with FCS. However, a comparable response was induced when CS or NBS was added at 1% to the culture medium in combination with 500 μg/ml β-cyclodextrin (β-CD). In the presence of β-CD, the response was the highest when CS or NBS was added at 1%. An important observation was that all the lots of CS (5 lots) and NBS (2 lots) tested supported the response equally in the presence of β-CD. The anti-sheep erythrocytes response induced by 1% CS or NBS in the presence of β-CD was shown to be completely dependent on the added antigen, suggesting that β-CD is not a polyclonal activator. β-CD showed neither mitogenic nor cytotoxic effects on murine lymphocytes. These results indicate that β-CD is a useful additive for inducing the primary antibody response in vitro in the culture medium containing CS or NBS which is usually less supportive but less expensive than FCS.

**Keywords** — in vitro antibody response; calf serum; newborn bovine serum; β-cyclodextrin; serum substitute

INTRODUCTION

In order to induce the primary antibody response in vitro, murine lymphocytes are usually cultured with antigen in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS). It has been reported that the response varies drastically depending on the lot of FCS used in the culture medium. According to the report by Shiigi and Mishell, less than 10% of all lots of FCS tested were fully supportive (good lot), but the remaining 90% were less active (deficient lot). Therefore, it is difficult to obtain good lots of FCS for laboratory use. If the antibody response can be induced with a reduced amount of serum, or can be elicited even with deficient lots of serum, such a procedure would be valuable in various experimental systems. In our previous paper, we reported that β-cyclodextrin (β-CD), α-1,4-cyclohexaamylose (Fig. 1), is useful as a serum-substitute in inducing the primary antibody response to sheep erythrocytes (SRBC) in vitro in murine lymphocytes. In the presence of 500 μg/ml β-CD and 1% FCS, we obtained a comparable response to that supported by 10% FCS. More importantly, all lots of FCS tested, whether they were good or deficient, supported the response almost equally when they were added to the culture medium at 1% in combination with β-CD. On the other hand, calf serum (CS) or newborn bovine serum (NBS), al-

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FIG. 1. Structure of β-CD
though they are less expensive than FCS, are not usually used in lymphocyte cultures, probably because they are deficient in supporting the antibody response. In the present paper, we report that the combination of CS or NBS with β-CD is useful for eliciting the antibody response as efficiently as FCS.

MATERIALS AND METHODS

Mice — Female BALB/c mice (6–8 weeks of age) were purchased from Shizuoka Cooperation of Experimental Animals (Shizuoka, Japan), and were used during 10–15 weeks of age.

Materials — Chemicals and various materials were obtained from the following sources; 2-mercaptoethanol (2-ME) (Tokyo Chemical Industry, Tokyo, Japan), SRBC (Nishinippon Sheep Farm, Fukuyama, Japan), FCS, NBS and CS (GIBCO, Grand Island, NY and Flow Laboratories, McLean, VA, U.S.A.), lipopolysaccharide (LPS) of Escherichia coli 055 B5 (Difco Laboratories, Detroit, MI, U.S.A.), Concanavalin A (Con A) (E. Y. Laboratories, San Mateo, CA, U.S.A.), [3H]thymidine ([3H]Tdr) (RCC Amer sham, England) and RPMI-1640 medium (Nissui Seiyaku, Tokyo, Japan). β-CD was a gift from Dr. Y. Suzuki of Teijin Institute for Biomedical Research. It is also commercially available from various distributors. RPMI-1640 medium was usually supplemented with 100 μg/ml streptomycin and 100 unit/ml penicillin G.

Primary Antibody Response in Vitro — Murine lymphocytes were cultured as described previously. Briefly, BALB/c spleen cells (6 × 10⁶) were cultured in duplicate or triplicate with 2 × 10⁸ SRBC in 1 ml of RPMI-1640 medium containing 10⁻⁵ M 2-ME and 0–10% FCS, CS or NBS. Where indicated, β-CD was added. Cultures were incubated at 37 °C for 5 d in a humidified atmosphere of 5% CO₂ and 95% air using plastic culture plates with 24 wells (NUNC, Kamstrup, Denmark). The anti-SRBC response was assayed by enumerating direct plaque-forming cells (PFC) by the method of Jerne and Nordin. Typical data from several repeated experiments were presented and expressed as the mean number of PFC or the mean number of PFC + standard error. The experimental variations among multiple cultures did not exceed 15% of the mean values.

Proliferative Responses in Murine Lymphocytes — BALB/c spleen cells (1 × 10⁶) were cultured in triplicate with β-CD (100–500 μg/ml), LPS (25 μg/ml) or Con A (1 μg/ml) in 0.2 ml of RPMI-1640 medium containing 1% CS and 10⁻⁵ M 2-ME at 37 °C for 72 h under 5% CO₂ and 95% air. Then, the cells were pulsed with 0.5 μCi of [3H]Tdr for 18 h, and harvested onto a glass filter using an automatic cell harvester. The incorporated radioactivities were measured by a liquid scintillation counter. Two lots of NBS and 5 lots of CS were tested in this study. NBS-1 and CS-4, as designated in Fig. 7, were used in the experiments shown in Figs. 3–6 and Table I.

RESULTS

The in vitro antibody response to SRBC in murine lymphocytes was diminished when the FCS content was reduced to 1% in RPMI-1640 medium as shown in Fig. 2. The response was, however, restored to the level in 10% FCS-containing medium when β-CD was supplemented to 1% FCS-containing medium as reported previously. The addition of β-CD did not affect the immune response in the presence of 10% FCS. We then tested whether the antibody response could be induced efficiently with CS or NBS instead of FCS when the culture

![FIG. 2. β-CD Is Effective in Inducing the Primary Antibody Response in Vitro under FCS-Limited Conditions](image-url)

BALB/c spleen cells (6 × 10⁶) were cultured with 2 × 10⁸ SRBC in 1 ml of RPMI-1640 medium containing 1 or 10% FCS in the presence of 10⁻⁵ M 2-ME for 5 d. Where indicated, 500 μg/ml β-CD was added.
FIG. 3. Induction of the Primary Antibody Response to SRBC in Vitro Using NBS or CS in the Presence of β-CD

BALB/c spleen cells (6 × 10⁶) were cultured with 2 × 10⁶ SRBC in 1 ml of RPMI-1640 medium containing 10⁻⁵ M 2-ME and 0—10% NBS or CS for 5 d in the presence (●) or absence (○) of 500 μg/ml β-CD as described in Materials and Methods. The horizontal broken line shows the level of the response supported by 10% FCS.

FIG. 4. One Percent NBS or CS Is Required for the Induction of an Optimal Antibody Response in the Presence of β-CD

BALB/c spleen cells (6 × 10⁶) were cultured with 2 × 10⁶ SRBC in 1 ml of RPMI-1640 medium containing 0—1% NBS or CS in the presence of 500 μg/ml β-CD as described under Materials and Methods. The horizontal broken line represents the level of the response supported by 10% FCS.

FIG. 5. Dose-Response Profile of β-CD in Inducing the Primary Antibody Response in the Presence of 1% NBS or CS

Murine spleen cells (6 × 10⁶) were cultured with 2 × 10⁶ SRBC in 1 ml of RPMI-1640 medium containing 1% NBS or CS in the presence of varying concentrations of β-CD as described under Materials and Methods.
have mitogenic effects on the lymphocytes under the culture conditions of the antibody response because β-CD alone did not induce a significant uptake of [3H]TdR by murine lymphocytes while LPS and Con A elicited proliferative responses (Table I).

Finally, 2 lots of NBS and 5 lots of CS were tested for their capacities to support the primary antibody response when they were added at 1% to the culture medium in combination with 500 μg/ml β-CD. Under these experimental conditions, all lots of CS or NBS tested were found to induce the response as effectively as a good lot of FCS (Fig. 7B), although CS or NBS showed much weaker activities than FCS when these sera were added at 10% as shown in Fig. 7A.

The above results clearly showed that CS or NBS can be used in place of FCS for eliciting the primary antibody response in vitro provided that β-CD was added to the culture medium.

**DISCUSSION**

In the previous paper,5) we have reported the following points. 1) In the presence of β-CD, FCS that is usually added at 10% to the culture medium could be reduced to 1% for inducing an optimal antibody response in vitro in murine lymphocytes. 2) Even a deficient lot of FCS gave the response comparable to that supported by a good lot of FCS when it was added at 1% in combination with β-CD. 3) β-CD did not show mitogenicity, cytoxicity and activity of a polyclonal activator. In the present paper, similar observations were made when FCS was replaced by CS or NBS. We have confirmed that the antibody response in the culture medium containing β-CD plus CS or NBS was completely dependent on the added antigen. Therefore, the response

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**TABLE I. β-CD Does Not Show a Mitogenic Effect on Murine Lymphocytes**

<table>
<thead>
<tr>
<th>Addition to the culture</th>
<th>Dose (μg/ml)</th>
<th>[3H]TdR uptake (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-CD</td>
<td>0</td>
<td>631 ± 22</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>417 ± 111</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>399 ± 79</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>714 ± 90</td>
</tr>
<tr>
<td>LPS</td>
<td>25</td>
<td>2359 ± 323</td>
</tr>
<tr>
<td>Con A</td>
<td>1</td>
<td>10928 ± 1048</td>
</tr>
</tbody>
</table>

BALB/c spleen cells (1 × 10⁶) were cultured in 0.2 ml of RPMI-1640 medium containing 1% CS and 10⁻⁵ M 2-ME in the presence of the indicated agents as described under Materials and Methods.
obtained under these conditions was not due to nonspecific stimulation of the lymphocytes. The above data suggest that $\beta$-CD is a useful additive for utilizing CS or NBS that is otherwise deficient in supporting the antibody response in vitro. The use of CS or NBS in the lymphocyte culture would be valuable from an economical point of view. The important point was that any lot of CS or NBS tested could support the antibody response equally well in the presence of $\beta$-CD.

$\beta$-CD has been known to form inclusion complexes with various hydrophobic compounds. On the other hand, the role of the serum in the cell culture is not only to supply various nutrients or growth factors but also to neutralize inhibitory factors in the culture medium by adsorbing them to macromolecular components like albumin as pointed out by Ham and Mckeehan. The latter role of the serum might be replaced by $\beta$-CD. As indicated in the previous paper, maltoheptaose, a linear analogue of $\beta$-CD, that lacks the ability to form inclusion complexes was inactive. Therefore, the ability to form inclusion complexes would be responsible for its stimulating effect. However, it remains unclear what the targets of $\beta$-CD are. Imaizumi et al. have reported that the production of *Bordetella pertussis* toxin was dramatically enhanced by the addition of heptakis (2,6-O-dimethyl) $\beta$-CD to a synthetic culture medium in which the toxin production was negligible. The effect of the additive in this case appears to be due to the formation of inclusion complexes with unsaturated fatty acids that are inhibitory for the growth of *B. pertussis*. A similar mechanism might be involved in the case of murine lymphocyte culture, although the direct interaction of $\beta$-CD with some populations of the lymphocytes can not be ruled out. The fact that the stimulatory effects of $\beta$-CD were abolished in the medium containing 10% CS or NBS could be due to the presence of the excess amount of factors that saturate added $\beta$-CD.

It was shown that an optimal antibody response was elicited with 1% serum in the presence of $\beta$-CD, thus suggesting all nutrients and growth factors required for the lymphocyte proliferation and differentiation can be supplied from that amount of the serum. It has not been revealed what components in the serum are really essential for the induction of the primary antibody response. Burger has reported that FCS can be replaced by fetuin in this system. Mosier, however, has stated in his paper that he could not induce the primary antibody response to trinitrophenyl-Ficol in the fetuin-containing medium reported by Burger. It would be expected that the analysis of the essential components in the serum by employing $\beta$-
CD-containing culture system would be useful for the establishment of serum-free culture conditions.

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