STUDIES ON THE MECHANISM OF ACTION OF DIKETOCORIOLIN B TO ENHANCE ANTIBODY FORMATION

MASAAKI ISHIZUKA, TOMIO TAKEUCHI and HAMAO UMEZAWA

Institute of Microbial Chemistry, 14-23 Kamiosaki 3-Chome, Shinagawa-ku, Tokyo 141, Japan

(Received for publication July 21, 1980)

Diketocoriolin B (DKC), at a very low dose (0.1 μg/mouse) or in a low concentration (0.01 ng/culture) augmented antibody formation to sheep red blood cells (SRBC) *in vivo* and *in vitro*. The addition of DKC to spleen cell cultures 24~48 hours after the start of the culture showed the strongest effect. The stimulatory effect was not influenced by the elimination of macrophages. DKC also augmented antibody formation in T cell-depleted spleen cell cultures and in cultures of spleen cells of athymic mice. The treatment of spleen cells with DKC at 37°C or 4°C for 30 minutes before the start of the culture enhanced antibody formation to SRBC. Both treatments of macrophage-rich and lymphocyte-rich cells with DKC enhanced antibody formation.

Diketocoriolin B is an active derivative of coriolin B produced by *Coriolus consors*. It inhibits the growth of some Gram-positive bacteria, EHRLICH ascites carcinoma and L-1210[1]. The mechanism of its cytotoxic action has been reported to be due to the inhibition of Na-K-ATPase, a membrane-associated enzyme[2]. Moreover, as reported in a previous paper[3], it enhances antibody formation in mice to sheep red blood cells.

In this paper, we report the mechanism of action of diketocoriolin B in enhancing antibody formation.

Materials and Methods

Animals

Female *dd/Y* (6~8 weeks old) and female C57B1/6 (6~8 weeks old) mice were obtained from Shizuoka Laboratory Animal Agriculture Cooperative Association, Shizuoka, Japan. Female CDF_1 mice, 6~8 weeks old, were supplied by the Institute of Medical Science, University of Tokyo. Athymic mice (Nu/Nu, Balb/c) and their litter mates (Nu/+, Balb/c) were supplied by Dr. T. NOMURA, Central Institute for Experimental Animals. They were housed in sterilized cages and fed sterilized mouse pellet (MB-1, Funabashi Farm Co., Ltd., Chiba, Japan) and water *ad libitum*.

Albino rabbits (female, 2~3 kg) were obtained from Funabashi Farm Co.

Antigen

Sheep red blood cells (SRBC) obtained in ALSEVER's solution from Funabashi Farm Co., Chiba, Japan, were washed with saline and counted with the use of a hemocytometer.

Diketocoriolin B

The method of preparation and the structure of diketocoriolin B (DKC) have been reported in a previous paper[1]. DKC was dissolved in dimethylsulfoxide (DMSO) at various concentrations (5.0~10.0 mg/ml), and, with a trace of Tween 80, was diluted with saline or a culture medium to the desired concentration. The carrier medium (0.05 ml of the medium containing $2\times10^{-3}\sim2\times10^{-7}$% of DMSO (v/v) and with a trace amount of Tween 80), employed to dissolve DKC, did not show any effect on antibody formation.

Preparation of rabbit anti-mouse brain θ serum

Rabbit anti-mouse brain θ serum (anti-BAθ serum) was prepared by the method of GOLUB[4]. Five brains of C57Bl/6 mice were homogenized in 3.0 ml of balanced salt solution with a loose glass homogenizer and the homogenate was mixed with an equal volume of complete FREUND's adjuvant. One ml of the mixture was injected subcutaneously into 3 or 4 sites in a rabbit. One week thereafter, a booster immunization was performed. The serum was collected 7 days after the booster and frozen at $-20°C$. The antiserum was heated at 56°C for 30 minutes and absorbed with mouse erythrocytes prior to use.

Antibody formation in mice

The effect of DKC on primary antibody formation was tested as follows: *dd/Y* mice were immunized by intravenous injection of 10^8 SRBC; at the same time 0.1 μg DKC in 0.25 ml of saline was given intraperitoneally. The control group received 0.25 ml of saline. Antibody formation was measured by counting plaque-forming cells (PFC)[5,6] in spleen on each day after the immunization. Each group consisted of 5 mice.

In experiments to test the effect of DKC on secondary antibody formation, *dd/Y* mice were primed by the intravenous injection of 10^5 SRBC and, at the same time, DKC was given intraperitoneally. The control group was given saline. Antibody formation was checked 22 days later; on the following day, secondary immunization was carried out by the intravenous injection of 10^5 SRBC. Four days thereafter, antibody-forming cells were enumerated.

Spleen cell culture for antibody formation *in vitro*

The procedure described by MISHELL and DUTTON[7] was employed to examine primary antibody formation in mouse spleen cell suspensions prepared from CDF_1 mice or athymic mice. Spleen cells in cold HANKS balanced salt solution (HBSS) were centrifuged at $600 \times g$ for 5 minutes and, after the supernatant fluid was discarded, the sedimented cells were resuspended to 1.5×10^7 cells/ml in EAGLE's minimum essential medium (MEM) supplemented with sodium pyruvate, non-essential amino acids, glutamine and 10% fetal calf serum (Lot 438 C, Colorado Serum Co., Denver, Col., U.S.A.). One ml of the cell suspension in a plastic dish (Falcon 3001, Div, Becton, Dickinson and Co., Oxnard, Calif., U.S.A.) was incubated in a humid atmosphere of 5% CO_2 at 37°C on a Bellco oscillating platform (Bellco Glass Inc., Veneland, N. J., U. S. A.). As the antigen, 10^6 SRBC in 0.05 ml were added at the initiation of the culture. After four days, antibody formation was measured by counting plaque-forming cells.

T cell-depleted cultures were prepared as follows. Freshly harvested spleen cells were suspended in a 1: 100 dilution of rabbit anti-BAθ serum at 5×10^7 cells/ml, incubated at 4°C for 30 minutes, washed twice with 40 ml of HBSS, and resuspended in a 1: 10 dilution of guinea pig complement. The cells were incubated at 37°C for 30 minutes, washed twice with HBSS and resuspended in the culture medium described above. Each culture received 10^6 SRBC and was incubated at 37°C in 5% CO_2 and air; 4 days later, the PFC were determined. Triplicate cultures were made for each datum.

Spleen cells from CDF_1 mice were divided into two cell populations, macrophage-rich (MR) and lymphocyte-rich (LR) cells[8,9]. MR (1×10^6 cells/ml), which was the adherent cell population on a plastic dish (Falcon 3001), was cultured with 10^6 SRBC and 0.01 ng/ml DKC at 37°C for 1 hour in 5% CO_2. LR (1×10^7 cells/ml), which was the non-adherent cell population, was cultured with 0.01 ng/ml DKC at 37°C for 1 hour in 5% CO_2. After the incubation, each cell population was washed thoroughly with serum-free MEM and the DKC in the LR culture or the DKC and SRBC in the MR culture were removed. Each cell population was then suspended in fresh culture medium. LR (1×10^7 cells) and MR (10^6 cells) were mixed and the reconstituted mixture was incubated at 37°C in 5% CO_2 and air; 4 days later, the number of PFC was determined.

Results

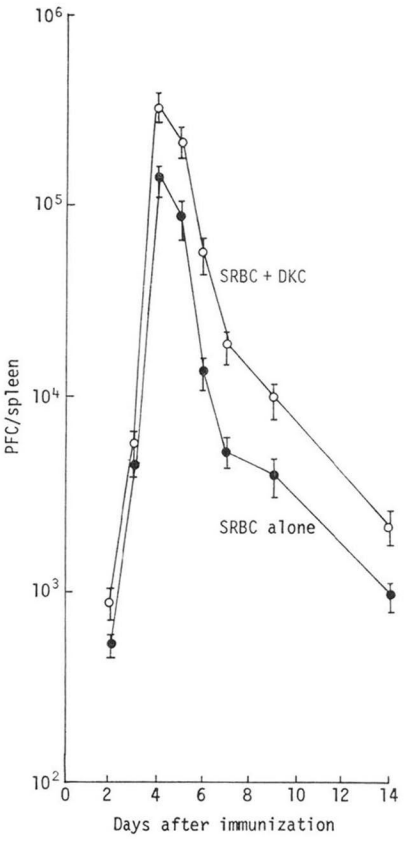
Effect of DKC on Primary and Secondary Antibody Formation in Mice

As reported in a previous paper[3], DKC stimulates primary and secondary antibody formation to SRBC in mice. This was further studied in detail. Mice were immunized by the intravenous route with

10^8 SRBC with or without the simultaneous intraperitoneal injection of 0.1 μg of DKC. The number of direct plaque-forming cells (PFC) was counted from 2 to 14 days after immunization. As shown in Fig. 1, the number of PFC increased following DKC administration. The stimulatory ratios on the various days after the immunization were as follows: 1.73 after 2 days, 1.37 after 3 days, 2.26 after 4 days, 2.45 after 5 days, 3.89 after 6 days, 3.37 after 7 days, 2.41 after 9 days and 2.32 after 14 days.

Fig. 1. Effect of diketocoriolin B on primary IgM antibody formation to SRBC in mice.

Mice were immunized by intravenous injection of 10^8 SRBC and injected intraperitoneally with 0.1 μg of diketocoriolin B (DKC) in 0.2 ml at the same time.



The effect of DKC on the secondary response was tested in mice which had been primed by intravenous injection of 10^5 SRBC with or without the simultaneous intraperitoneal injection of 0.1 μg or 0.01 μg of DKC. Twenty two days later, the number of PFC in the primed mice was almost the same as or was lower than that of the normal control without antigen administration. On the following day, secondary immunization with 10^5 SRBC was given, and, 4 days later, the number of PFC was determined. As shown in Table 1, the injection of 0.1 μg or 0.01 μg of DKC with the priming SRBC increased the number of PFC observed in the secondary response by more than 10-fold.

Effect of DKC on Primary Antibody Formation *In Vitro*

The influence of DKC on primary antibody formation *in vitro* was examined by the method of MISHELL and DUTTON[7]. The effect of various doses of DKC added at the start of the culture is

Table 1. Effect of diketocoriolin B on secondary antibody formation to SRBC in mice*.

Primary immunization	PFC/spleen 22 days after the primary injection	Second immunization on day 23	PFC/spleen 4 days after the secondary injection
SRBC 10^5 i.v.	23.3±11.7	—	21.3± 3.6
〃	—	SRBC 10^5	1,816.7± 195.2
〃 +DKC 0.1μg i.p.	22.5± 7.2	〃	25,000.0±2,235.2
〃 +DKC 0.01μg i.p.	18.3± 6.7	〃	21,750.0±3,240.3
—	18.3± 4.4	〃	16.3± 3.0
—	—	—	21.3± 5.1

* Mice were primed by intravenous injection of 10^5 SRBC and were given intraperitoneally 0.1 or 0.01 μg of diketocoriolin B (DKC) in 0.2 ml at the same time. After twenty three days, 10^5 SRBC was injected intravenously and 4 days later the number of PFC was enumerated.

Fig. 2. Effect of the concentration of diketocoriolin B on primary antibody formation *in vitro*.

Diketocoriolin B (DKC) in 0.05 ml was added to spleen cell cultures at the start of the incubation, and 4 days later the number of PFC was counted. Data represent the mean of PFC per 10^6 cells recovered from triplicate incubations.

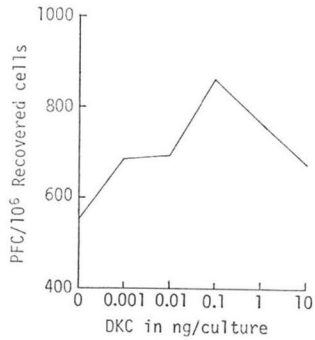


Table 2. Effect of diketocoriolin B added at various times during the course of incubation on antibody formation *in vitro*.*

DKC addition	PFC/10^6 on day 4	Ratio
SRBC	395.0	1.00
〃 + 0 hr.	653.8	1.66
〃 +24 hrs.	856.2	2.17
〃 +48 hrs.	1,266.9	3.21
〃 +72 hrs.	1,080.6	2.74

* 0.1 ng of diketocoriolin B (DKC) in 0.05 ml was added at 0, 24, 48 or 72 hours after the start of the culture. The number of PFC was determined 4 days after the start of the culture. Data represent the mean of PFC per 10^6 cells recovered per triplicate cultures. The standard deviation did not exceed 5%.

shown in Fig. 2. The addition of DKC at very low concentrations (0.001 ~ 10 ng/culture) increased the number of PFC, and the maximum increase was obtained with 0.1 ng. Therefore, DKC was added at 0.1 ng/culture in the following experiments, unless otherwise noted.

In order to determine the optimum time for the addition of DKC, the drug was added at 0, 24, 48 or 72 hours after the start of the incubation and the number of PFC was measured 96 hours later. As shown in Table 2, the maximum stimulatory effect of DKC was observed when the addition was made at 48 hours. Moreover, its addition even at 72 hours was more effective than that at 0 or 24 hours. These results indicate that DKC affects a late stage of antibody formation, possibly the differentiation of B cells into antibody forming cells[10].

Effect of DKC on Antibody Formation *In Vitro* by Adherent Cell-depleted Spleen Cell Cultures

To determine whether DKC acts on macrophages to increase the number of antibody-forming cells, spleen cells were cultured with SRBC for 24 hours: Cell clusters in the culture were dispersed with a Pasteur pipette and maintained for 2 hours in the incubator without rocking. Thereafter, the supernatant containing non-adherent cells was transferred to a fresh dish and further cultured for 72 hours. In one group, DKC was added at the initiation of the culture and in the second group, after the elimination of adherent cells. As shown in Table 3, the removal of adherent cells did not affect the action of DKC. DKC stimulated antibody formation in all cases; this suggests that the enhancing effect of adding DKC late in the incubation may be due to the activation of B cells.

Effect of DKC on Antibody Formation in T Cell-depleted Cultures and in Athymic Mice Spleen Cell Cultures

Spleen cells were treated with anti-BAθ serum, and guinea pig complement, and then, SRBC were added. At 0, 24 and 48 hours after the start of the culture, 0.1 ng of DKC was added. As shown in Table 4, antibody formation was not reduced by treatment with either anti-BAθ serum or complement alone, but it was markedly reduced when both were employed. The addition of DKC at 24 or 48 hours to the culture treated with anti-BAθ serum and complement restored antibody formation to normal levels.

The results described above indicate that DKC enhanced the antibody-forming ability of T cell-

Table 3. Influence of adherent cells on the stimulation of antibody formation *in vitro* by diketocoriolin B.

Addition to cultures	PFC/10^6 recovered cells*	
	Whole cells**	Without adherent cells***
SRBC	395.0	548.3
〃 +DKC 0 hr.	653.8	784.9
〃 +DKC 24 hrs.	856.2	1,308.7

* Data represent the mean number of PFC per triplicate cultures. The standard deviation did not exceed 5%.

** Spleen cells were cultured: 0.1 ng of diketocoriolin B (DKC) was added at 0 hour or at 24 hours after the start of the incubation.

*** This group was cultured for 24 hours on a rocking platform. Then, cell clusters were dispersed with a Pasteur pipet and the dispersed cells were incubated for an additional 2 hours. Non-adherent cells with the culture fluid were transferred to a fresh dish. DKC was added at the start of the incubation of the non-adherent cells or 24 hours thereafter.

Table 4. Effect of diketocoriolin B on antibody formation by T cell-depleted spleen cell culture.

Pretreated with*	Addition to cultures**	PFC/10^6 cells***
	SRBC	214.3
Anti-BAθ serum alone	〃	207.6
Complement alone	〃	203.3
Anti-BAθ serum and complement	〃	69.7
〃	〃 +DKC 0 hr.	85.8
〃	〃 +DKC 24 hrs.	223.1
〃	〃 +DKC 48 hrs.	212.8

* Spleen cells (5×10^7 cells/ml) were pretreated (1) with anti-BAθ serum (1 : 100) at 4°C for 30 minutes, (2) with anti-BAθ serum and guinea pig complement, or (3) with guinea pig complement alone (1 : 10) at 37°C for 30 minutes. After the pretreatment the cells were washed thoroughly and resuspended in the medium to give 1.5×10^7 cells/ml per culture.

** As the antigen, 10^6 SRBC in 0.05 ml were added to each culture at the start of the incubation. To the cultures pretreated with anti-BAθ serum and complement, 0.1 ng of diketocoriolin B (DKC) was added at 0, 24 or 72 hours after the start of the incubation.

*** The number of PFC was determined 4 days after the start of the culture. Data represent the mean number of PFC/10^6 cells recovered. The standard deviation did not exceed 5%.

depleted spleen cells. Consequently the effect of DKC on antibody formation by cultures of spleen cells derived from athymic mice was examined. DKC was added to cultures at 0, 24 and 48 hours after the start of the culture. As the normal control, antibody formation by spleen cell cultures from their litter mates was also tested. As demonstrated in Table 5, only a few antibody-forming cells were produced in spleen cell cultures derived from athymic mice in comparison with the normal control. The addition of DKC increased markedly the number of antibody-forming cells. In the case of both the normal and athymic mice, the maximal stimulatory effect of DKC was obtained when the compound was added after 24-hours incubation.

These results indicate that DKC stimulates antibody formation in T cell-deficient spleen cell cultures. Further, the data suggest that one important mechanism of action of DKC in augmenting antibody formation may be due to a stimulation of the proliferation of B cells and/or differentiation of B cells into antibody-producing cells.

Antibody Formation in Spleen Cells Pretreated with DKC

We next examined the effect of binding of DKC to immunocompetent cells. Whole spleen cells (15×10^6) were mixed with 0.1 ng of DKC in HBSS and cultured at 37°C or 4°C for 30 minutes. Then, the cells were washed twice with a large volume of HBSS, suspended in a culture medium and incubated

Table 5. Effect of diketocoriolin B on antibody formation by spleen cell cultures of athymic mice* *in vitro*.

Addition to cultures	PFC/10^6 recovered cells	
	Nu/+	Nu/Nu
10^6 SRBC	269.3	17.0
〃 +DKC 0 hr.	648.4	106.6
〃 +DKC 24 hrs.	1,003.2	187.2
〃 +DKC 48 hrs.	635.1	74.8

* Spleen cells of Nu/+ and Nu/Nu were cultured with 10^6 SRBC and 0.1 ng of diketocoriolin B was added at 0, 24 and 48 hours after the start of the incubation. The number of PFC was determined on day 4. Data represent the mean number of PFC/10^6 cells recovered from triplicate cultures. The standard deviation did not exceed 8 %.

Table 6. Antibody formation in diketocoriolin B-treated spleen cell cultures*.

Treated with	Addition	PFC/10^6 cells
None	SRBC	239.8
DKC 37°C for 30 min.	〃	554.2
〃 4°C 〃	〃	574.2

* Spleen cells (5×10^7 cells/ml) were treated with 0.1 ng of diketocoriolin B (DKC) per 1.5×10^7 cells at 37°C or 4°C for 30 minutes. Thereafter, the cells were washed thoroughly, and resuspended in the culture medium at 1.5×10^7 cells/ml per culture. The number of PFC was determined 4 days after the start of the culture. Data represent the mean number of PFC from triplicate cultures. The standard deviation did not exceed 5 %.

Table 7. Antibody formation in the cultures reconstituted from diketocoriolin B-treated macrophage-rich (MR) and lymphocyte-rich cell (LR) populations of spleen cells.

Cells reconstituted from*		PFC/10^6 recovered cells**	Ratio
MR-SRBC	—	0	—
〃	LR	440	1.00
〃	LR-DKC	1,050	2.39
MR-SRBC-DKC	—	0	—
〃	LR	710	1.61
〃	LR-DKC	1,390	3.16
—	LR	80	—
—	LR-DKC	20	—

* Macrophage-rich (MR) and lymphocyte-rich (LR) cell populations were prepared from spleen cells of normal mice; each cell population was incubated with 0.1 ng of diketocoriolin B (DKC)/ml. MR was also incubated with SRBC as antigen. One hour later each cell group was washed to remove DKC. After washing, the cell mixtures consisting of LR and MR were reconstituted and cultured for 4 days.

** The number of PFC was determined 4 days after the start of the culture. Data represent the mean number of PFC of triplicate cultures. The standard deviation did not exceed 8 %.

with SRBC for 4 days. As seen in Table 6, the pretreatment of spleen cells with DKC at both temperatures stimulated antibody formation, suggesting that DKC binds to lymphoid cells and enhances antibody formation.

Effect of DKC Pretreatment upon Different Spleen Cell Populations

In order to determine which population of spleen cells is activated by pretreatment with DKC, the cells were divided into macrophage-rich (MR) and lymphocyte-rich (LR) populations[8,9]. Each cell population was then pretreated with DKC. To the MR culture 10^6 SRBC and DKC were added at the same time. Each cell population was washed thorougly, and was mixed as follows: DKC-treated MR+DKC-nontreated LR; DKC-nontreated MR+DKC-treated LR; DKC-treated MR+DKC-treated LR; DKC-nontreated MR+DKC-nontreated LR. The mixed cells were cultured for 4 days, and the number of PFC was determined. As shown in Table 7, pretreatment of MR and LR with DKC augmented antibody formation. The maximum stimulatory effect was observed in the culture reconstituted from DKC-treated -MR and -LR. This indicates that DKC provided at the start of the culture, activates both lymphocytes and macrophages.

Discussion

DKC, at very low doses (0.1 μg~0.01 μg/mouse), augments antibody formation. In the case of antibody formation after primary immunization, DKC, given at the time of immunization, produced a 2- or 3-fold increase of antibody-forming cells, whereas, in the case of the secondary response, DKC, given at the time of the primary immunization, produced about a 10-fold increase of antibody-forming cells. It appears that DKC may be useful in the analysis of the secondary immune response or, the behavior of memory B cells[11].

When DKC was added to whole spleen cell cultures using SRBC for primary antibody formation *in vitro*, the optimum stimulatory effect of DKC was observed when the addition was made 24 or 48 hours after the start of the culture. Moreover, the stimulatory effect was not reduced by the elimination of adherent cells including macrophages as antigen presenting cells. Antibody formation, reduced by depletion of T cells, was restored by the addition of DKC at 24 or 48 hours after the start of the culture. DKC also enhanced antibody formation *in vitro* by spleen cells obtained from athymic mice. The results, obtained in the *in vitro* studies, thus indicate that the action of DKC is directed toward B cells either through an activation of B cell proliferation or their differentiation into antibody-producing cells.

There are two possible reasons to account for B cell activation; (1) the production of T cell-replacing factors[12~14] or B cell-activating factor[15,16] which are produced by T cells and/or macrophages[12,15,16] and (2) the B cells are directly activated[17]. The stimulation observed following the addition of DKC to spleen cell cultures does not require the presence of T cells and macrophages. Therefore, it appears most likely that DKC interacts directly with B cells.

The pretreatment of spleen cells with DKC before the stimulus of antigen also enhanced antibody formation. This effect was not influenced by the temperature of the incubation (4°C, 37°C) for 30 minutes nor by thorough washing of the cells to remove DKC indicating that DKC binds to immunocompetent cells. Moreover, as shown in Table 7, DKC activated both adherent cells including macrophages and non-adherent cells containing T and B cells. As reported by KUNIMOTO *et al.*[2], DKC inhibits NA-K-ATPase which is a membrane-associated enzyme; therefore, it seems likely that DKC binds to cell membranes. DKC is a small molecular weight compound which might be useful in the analysis of immune responses.

Acknowledgement

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan and the contract No. NO1-CM-57009 from the Division of Cancer Treatment, the National Cancer Institute, U. S. A.

References

1) TAKEUCHI, T.; S. TAKAHASHI, H. IINUMA & H. UMEZAWA: Diketocoriolin B, an active derivative of coriolin B by *Coriolus consors*. J. Antibiotics 24: 631~635, 1971
2) KUNIMOTO, T.; M. HORI & H. UMEZAWA: Mechanism of action of diketocoriolin B. Biochim. Biophys. Acta 298: 513~525, 1973
3) ISHIZUKA, M.; H. IINUMA, T. TAKEUCHI & H. UMEZAWA: Effect of diketocoriolin B on antibody formation. J. Antibiotics 25: 320~321, 1972
4) GOLUB, E. S.: Brain-associated θ antigen: Reactivity of rabbit anti-mouse brain with mouse lymphoid cells. Cellular Immunol. 2: 353~361, 1971
5) JERNE, N. K.; A. A. NORDIN & C. HENRY: The agar plaque technique for recognizing antibody-producing cells. *in* Cell-bound antibodies, pp. 109~122. Wistar Institute Press, Philadelphia, 1963
6) CUNNINGHAM, A. J. & A. SZENBERG: Further improvements in the plaque technique for detecting single antibody-forming cells. Immunology 14: 599~600, 1968
7) MISHELL, R. I. & R. W. DUTTON: Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. 126: 423~442, 1967

8) MOSIER, D. E.: A requirement for two cell types for antibody formation *in vitro*. Science 158: 1573~1575, 1967
9) ISHIZUKA, M.; W. BRAUN & T. MATSUMOTO: Cyclic AMP and immune responses. I. Influence of poly A: U and cAMP on antibody formation *in vitro*. J. Immunol. 107: 1027~1035, 1971
10) PIERCE, C. W.: Immune responses *in vitro*. VI. Cell interactions in the development of primary IgM, IgG and IgA plaque-forming cell responses *in vitro*. Cellular Immunol. 9: 453~464, 1973
11) BROOKS, K. H.; T. L. FELDBUSH & A. VAN DER HOVEN: The generation of memory B-cell subpopulations capable of proliferation and antigen. Cellular Immunol. 50: 392~404, 1980
12) HOFFMAN, M. K. & J. WATSON: Helper T cell-replacing factors secreted by thymus derived cells and macrophages: Cellular requirements for B cell activation and synergistic properties. J. Immunol. 122: 1371~1375, 1979
13) JACOB, D. M.: Synergy between T cell-replacing factor and bacterial lipopolysaccharide (LPS) in the primary antibody response *in vitro*: A model for lipopolysaccharide action. J. Immunol. 122: 1421~1426, 1979
14) WATSON, J.; L. A. AARDEN, J. SHAW & V. PAETKAU: Molecular and quantitative analysis of helper T cell-replacing factors on the induction of antigen-sensitive B and T lymphocytes. J. Immunol. 122: 1633~1638, 1979
15) HOFFMAN, M. K.; S. KOENIG, R. S. MITTLER, O. P. RALPH, V. GALAROS & U. HAMMERLING: Macrophage factor controlling differentiation of B cells. J. Immunol. 122: 497~502, 1979
16) WOOD, D. D. & S. L. GAUL: Enhancement of the humoral response of T cell-depleted murine spleens by a factor derived from human monocytes *in vitro*. J. Immunol. 113: 925~933, 1974
17) WATSON, J. & C. WHITLOCK: Effect of a synthetic adjuvant on the induction of primary immune responses in T cell-depleted spleen cultures. J. Immunol. 121: 383~389, 1979