Human Lymphocyte Mitogen of Microbial Origin and Cell-Cell Interaction among Leukocytes in Response to the Mitogen

YASUO SETO,* KUNIO OISHI and KO AIDA

*Institute of Applied Microbiology, The University of Tokyo,
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan*

Received February 5, 1987

Mitogenic substances on human peripheral blood mononuclear leukocytes were screened from culture filtrates of microorganisms newly isolated from soil and sea water by measuring [3 H]-thymidine incorporation into the cells. Strong mitogenic activity was found in marine bacteria, particularly in marine vibrios. These mitogen samples exhibited neither hemagglutinating activity nor leucoagglutinating activity. They could scarcely stimulate murine lymphocytes.

Cell-cell interaction among leukocyte subsets in response to a bacterial mitogen was investigated using the most powerfully mitogenic sample (culture filtrate of strain H 52-2). A slight decrease in the mitogen response was observed on depletion of plastic surface adherent cells. Separation of T and non-T cells from each other by erythrocyte-rosette sedimentation resulted in a markedly diminished mitogen response. Considerable restoration of the mitogen response was obtained when T cells were mixed with mitomycin C-treated adherent cells or mitomycin C-treated non-T lymphocytes, or when non-T lymphocytes were mixed with mitomycin C-treated T cells.

Mitogens, which induce transformation in a polyclonal range of lymphocytes, are useful tools for the fundamental studies in immunology, *i.e.*, studies on the mechanism of lymphocyte activation or *in vitro* diagnosis of immune function. Some plant lectins¹⁾ are known to be mitogenic, and classified with respect to animal species specificity and proliferating cell specificity. Microorganisms were also found to produce mitogens such as lipopolysaccharides²⁾ (LPS) and the lymphocytosis-promoting factor of *Bordetella pertussis*.³⁾ Most of these mitogens, however, show high species specificity and act only on rodent lymphocytes. Therefore, clinical studies for elucidation of the human immune function lag far behind those concerning rodents.

The screening of mitogenic substances from microorganisms in nature has not yet been undertaken systematically. Recently, Ariizumi *et al.* reported the distribution of strains producing mitogens for murine lymphocytes among nonpathogenic microorganisms, and

precipitate of the culture filtrate of *Pseudomonas vesicularis* IAM 12105.⁴⁾ We have tried to obtain new mitogens for human lymphocytes from microorganisms and found much mitogenic activities in marine bacteria. In this paper, we describe the distribution of mitogen producers among newly isolated microorganisms and the cell-cell interaction mechanism of human leukocytes in response to the most potent microbial mitogen obtained.

MATERIALS AND METHODS

Materials. Lymphoprep and Ficoll-Paque were purchased from Nyegaard & Co. (Oslo, Norway) and Pharmacia (Uppsala, Sweden), respectively. Lipopolysaccharide (*E. coli* serotype 0111: B₄, phenol extract), neuraminidase (Type V) and concanavalin A (Con.A) (Type IV) were purchased from Sigma Chemical Co. (St. Louis). RPMI-1640 medium and fetal calf serum (FCS) were obtained from Grand Island Biological Co. (Grand Island, N.Y.). Pokeweed mitogen (PWM) and phytohemagglutinin (PHA) were obtained from E. Y.

* Present address: National Research Institute of Police Science, Sanban-cho, Chiyoda-ku, Tokyo 102, Japan.

Laboratory and Difco (Detroit, Michigan), respectively. [^3H]-Thymidine (20.0 Ci/mmol) was purchased from New England Nuclear (Boston, Mass.). Sheep blood was obtained from Japan Biosupply Center (Tokyo). All other chemicals were of analytical reagent grade.

Preparation of murine lymphocytes. C57BL/6 mice (4~12 week old, female) were purchased from Charles River Japan, Inc. (Atsugi). Their spleens were removed and teased out with forceps under sterile conditions. Free splenic cells were incubated at 37°C for 5 min in a solution containing 0.01 M Tris(hydroxymethyl)aminometane and 0.83% ammonium chloride (pH 7.4) to lyse contaminating erythrocytes (ACT treatment). Thymic cells were obtained as described for splenic cells.

Preparation of human mononuclear leukocytes. Human mononuclear leukocytes (MNL) were isolated from peripheral blood as described by Böyum.⁵ Venous blood from healthy adults was heparinized (100 IU/ml blood) and then left at room temperature for 10 min. Five ml of defibrinated blood was diluted twice with Dulbecco's phosphate-buffered saline (—)⁶ (PBS) and then layered upon 4 ml of Lymphoprep, followed by centrifugation (400 × g) at room temperature for 30 min. MNL at the interface were collected and washed with PBS by centrifugation (250 × g, 5 min).

Assay for mitogenic activity. Mitogenicity was assayed by measuring [^3H]-thymidine uptake according to the method of Janossy and Greaves.⁷ Isolated human MNL were suspended in RPMI-1640 medium supplemented with 10% human serum, 0.2% NaHCO_3 and 0.01% kanamycin, at a concentration of 5.0×10^5 living cells per ml. Two hundred μl of this suspension was applied to each well of a Microtest II plate (Falcon, Oxnard, Ca.), followed by culturing at 37°C in a humidified CO_2 incubator under a flow of 5% CO_2 in air. Five μl of a mitogen sample and 0.25 μCi of [^3H]-thymidine were added to the culture at 0 hr and 48 hr, respectively. After a further 24 hr culture, the cells were harvested on a glass fiber filter (Harvester Filter, Labo Science, Tokyo) and dried up. The radioactivity incorporated into the cells was measured with Packard 3255 liquid scintillation counter (Packard, Downers Grove, Illinois). The mean counts per minute (cpm) were determined for triplicate cultures. Mitogenic activity is expressed as a stimulation index (SI), which is the ratio of the radioactivity incorporated into cells in the presence of a mitogen sample to that in its absence. The average SI value (aSI) of a mitogenic sample was calculated with the following equation:

$$\log(\text{aSI}) = (1/n) \sum (\log \text{SI}_j)$$

where SI_j is the SI in a mitogenic assay for each MNL donor and n is the number of assays. In the experiment with fractionated cells described below, the average radioactivity incorporated into cells (aC) was calculated

with the following equation:

$$\log(\text{aC}) = (1/n) \sum (\log \text{C}_j)$$

where C_j is the radioactivity in each mitogenic assay and n is the number of assays.

The conditions for the mitogen assay for murine lymphocytes were identical to those for human MNL except for the cell concentration (2.5×10^6 cell per ml) and the serum used (FCS).

Fractionation of human mononuclear leukocytes. Human thymus-derived lymphocytes (T cells) were prepared according to the erythrocyte-rosette (E-rosette) method of Jondal *et al.*⁸ and Galili and Schledinger⁹ with some modification. Sheep red blood cells (SRBC) were treated with neuraminidase (final, 0.1 units/ml) at 37°C for 30 min and then suspended in Hank's balanced salt solution (HBSS) at a concentration of 1.5% (v/v). Immobilized FCS was adsorbed with SRBC (3 times, FCS:packed SRBC=9:1 (v/v), at room temperature for 30 min) to eliminate natural antibodies against SRBC. One volume of a human MNL suspension at a concentration of about 1×10^7 cells per ml of HBSS, two volumes of neuraminidase-treated SRBC (1.5%) and one volume of FCS were mixed, incubated at 37°C for 15 min, and then the mixture was spun at 200 × g for 2 min. The sediment was kept in ice for 1.5 hr, and then gently resuspended in HBSS, followed by layering upon the top of a Ficoll-Paque solution and then centrifugation at 450 × g for 30 min. The cells at the interface were collected and washed with PBS, and designated as 'T-depleted cells.' The pellet was subjected to ACT treatment and washed with PBS, and designated as 'T-enriched cells.'

Human macrophages and monocytes were prepared utilizing the property of their adherence to a plastic surface.¹⁰ A human MNL suspension at a concentration of 1.0×10^7 cells per ml of RPMI-1640 medium containing 10% FCS was incubated in a tissue culture dish (60 × 15 mm, Falcon) at 37°C under a flow of 5% CO_2 in air. During the incubation the dish was gently shaken twice. The floating cells were collected and washed with PBS, and designated as 'Nonadherent cells.' The adhesive cells in the dish were washed twice with warm medium, detached from the dish with a rubber policeman¹¹ and washed twice with PBS, and designated as 'Adherent cells.'

Hemagglutination assay. On a microtiter plate (Cooke Engineering Co., Alexander, Va.), one drop of a mitogen sample was added to an equal volume of 0.05 M phosphate-buffered saline (pH 7.0), followed by mixing with a drop of a 2~3% suspension of human, horse, chicken and rabbit erythrocytes with a micromixer (Kowakizai, Tokyo) for 1 min. After 60~120 min, hemagglutinating activity was determined by observation with the naked eyes.

Mitomycin C treatment. The separated leukocyte frac-

tion was incubated with mitomycin C (mmc) (25 µg/ml culture) at 37°C in a humidified CO₂ incubator under a flow of 5% CO₂ in air, and then washed three times with PBS.

Preparation of mitogen samples. The microorganisms examined were newly isolated and divided into four groups with respect to their habitats and the isolation procedures. The S-series bacteria were isolated from soil with trypto-soy medium (Eiken Chemical Co., Tokyo), the H and V-series bacteria from sea water with trypto-soy medium and thiosulfate-citrate-bile-sucrose (TCBS) medium (Eiken), respectively, and the A-series actinomycetes from soil with a medium (pH 7.0) containing 1% glucose, 0.4% Daigo Polypepton, 0.2% Ehrlich meat extract (Kyokuto), 0.2% yeast extract (Difco) and 2% agar. The S-series strains and the H and V-series strains were cultured with shaking at 30°C for 20 hr in a medium (pH 7.0) containing 3% peptone and 0.5% yeast extract in tap water, and in a mixture of sea and tap water (1:1, v/v), respectively. The A-series strains were cultured with shaking at 30°C for 48 hr in a medium (pH 6.0) containing 0.5% Polypeptone, 0.1% yeast extract, 1.5% lactose and 0.05% MgSO₄ in 0.05 M phosphate buffer. The culture broths were centrifuged at 10,000 × *g* for 10 min to remove cells, except for those of the A-series strains, which were filtered through a filter paper (Toyo-Roshi, No. 2). The resulting supernatants were filtered through a membrane filter (HA 0.45 µm, Millipore Japan, Tokyo) and then used directly as mitogen samples.

RESULTS

Screening of mitogen producing bacteria

The first screening of mitogen-producing strains was carried out with twelve divided

assays, each of which involved MNL from 12 different donors. Out of the culture filtrates of 419 strains (155 S-series, 96 V-series, 78 H-series and 90 A-series strains), 57 samples (21 S, 17 V, 13 H and 6 A) showed SI values exceeding 2. In the course of this screening, however, marked variation in the mitogen response of the test MNL to standard plant and microbial mitogens was observed. To avoid such variation due to differences in the MNL donors, a 2nd screening was carried out on the above 57 samples using MNL from three different donors with similar mitogen-responses, and in each assay the mitogenicity of selected samples was measured simultaneously. As shown in Table I, only ten samples showed average SI's exceeding 4, 8 of them being from H-series bacteria.

None of these 57 samples showed hemagglutinating activity toward human (blood types A, B and O), horse, chicken or rabbit erythrocytes or human leukocyte agglutinating activity, though the control plant lectins agglutinated human lymphocytes.

Finally we chose three strains, H 49-4, H 52-2 and H 79-1, as the best mitogen producers. These three were all isolated from sea water and grew well on TCBS medium. Table II shows the taxonomical properties of these strains. According to Cowan,¹¹⁾ the results suggest they are 'vibrios.'

TABLE I. OCCURRENCE OF HUMAN LYMPHOCYTE MITOGEN-PRODUCERS AMONG MICROORGANISMS

The [³H]-thymidine uptake in the absence of a mitogen sample was 894 ± 488 cpm (mean ± S.D.). The SI values for Con. A (25 µg/ml), PWM (1 µg/ml), PHA (1 µg/ml) and LPS (5 µg/ml) at the optimal dose were 53.7, 28.8, 95.5 and 1.57, respectively. The SI values for the media used for cultivating soil bacteria, marine bacteria and actinomycetes were 0.785, 1.01 and 0.885, respectively.

Microorganisms	1st screening selected	No. of mitogenic strains					
		average stimulation index (aSI)					
		1	~2	~3	~4	~5	~6
S-series	21	16	2	1	1	1	0
V-series	17	9	6	2	0	0	0
H-series	13	0	1	4	3	3	2
A-series	6	4	2	0	0	0	0
Total	57	29	11	7	4	4	2

Animal species specificity

The proliferative response of murine lymphocytes to the above three samples is shown in Table III. Con.A and LPS were used as standards of T cell mitogen and bone marrow derived lymphocyte (B cell) mitogen, respectively. Samples H 49-4, H52-2 and H 79-1 scarcely stimulated murine cells and sample H 52-2 was rather toxic for thymic cells. On the other hand, human MNL definitely proliferated in response to the three bacterial samples tested.

Mitogen response of human MNL fractionated by plastic adherence

To investigate the mechanism of the response of human MNL to a bacterial sample, human MNL were separated into two frac-

tions, adherent and nonadherent cells. Sample H 52-2 was used throughout the following studies because of its high mitogenic activity and constant production. As shown in Table IV, the nonadherent cell fraction responded to Con.A, PHA and PWM, similar to the unfractionated MNL. However, an apparent decrease in the mitogenic response to sample H 52-2 was seen. On the other hand, the adherent cells did not proliferate in response to Con.A or sample H 52-2.

Mitogen response of human MNL separated by erythrocyte-rosette sedimentation

As shown in Table V, a markedly diminished response of the separated cells to the bacterial sample was seen; the response being about 1/6 in T-enriched cells and 0 in T-

TABLE II. TAXONOMICAL PROPERTIES OF MITOGEN-PRODUCING BACTERIA ISOLATED FROM SEA WATER

Strain	H 49-4	H 52-2	H 79-1
Gram stain	—	—	—
Shape	Rod	Rod	Rod
Flagellation	Polar	Polar	Polar
Mobility	+	+	+
Effect of O ₂	F.A. ^a	F.A.	F.A.
OF test	— ^b	F	F
Catalase	+	+	+
MR test	—	+	—
V-P test	—	—	—
NO ₃ ⁻ reduction	+	+	+

^a Facultative anaerobic.

^b No effect on glucose.

TABLE III. RESPONSE OF MURINE AND HUMAN LYMPHOCYTES TO MITOGENIC SAMPLES

Each value represents SI. The [³H]-thymidine uptake in the absence of mitogens was 3470 cpm in murine splenic cells, 491 cpm in murine thymic cells and 417 cpm in human peripheral blood MNL.

	C57BL/6J mice ^a		Human ^b peripheral blood
	Spleen	Thymus	
Con. A (1 µg/ml)	16.4	32.9	53.7
LPS (25 µg/ml)	8.13	4.05	1.57
H 49-4 (5 µl)	1.59	1.15	7.75
H 52-2 (5 µl)	1.64	0.584	6.60
H 79-1 (5 µl)	1.59	1.68	4.72

^a Average SI values for 4 mice.

^b Average SI values for 7 donors.

TABLE IV. RESPONSE OF HUMAN PLASTIC SURFACE ADHERENT AND NONADHERENT CELLS TO MITOGENS

Each value represents the average radioactivity incorporated into cells (cpm).

	Con. A (25 µg/ml)	PWM (1 µg/ml)	PHA (1 µg/ml)	H 52-2 (5 µl)	Control ^d
Unfractionated ^a MNL	29,500	13,700	39,500	8,020	521
Nonadherent ^a	36,300	12,100	50,100	5,700	491
Adherent ^b	3,290	— ^c	— ^c	581	316

^a The average SI values for six experiments.

^b The average SI values for four experiments.

^c Not determined.

^d Values in the absence of mitogens.

TABLE V. RESPONSE OF HUMAN T-ENRICHED AND T-DEPLETED CELLS TO MITOGEN SAMPLES

Each value represents the average radioactivity incorporated into cells (cpm) in eight assays.

	Con. A (25 μ g/ml)	PWM (1 μ g/ml)	PHA (1 μ g/ml)	M 52-2 (5 μ l)	Control ^a
Unfractionated MNL	46,400	23,400	51,500	3,770	559
T-Enriched cells	36,300	14,500	42,400	1,060	428
T-Depleted cells	7,370	5,110	8,780	908	1,469

^a Values in the absence of mitogens.

depleted cells. On the other hand, the decreases in the responses of T-enriched and T-depleted cells proliferated in response to plant mitogens were not so great.

The mitogen response of T-enriched and T-depleted cells to sample H 52-2 was restored by mixing the two types of cells with each other (Figure 1). The highest response to sample H 52-2 was obtained with the mixture of T-enriched and T-depleted cells at a ratio of 75:25, with which [³H]-thymidine uptake was rather higher than that in the case of unfractionated MNL. This result rules out the possibility that the reduction in the mitogen response was due to a loss or inactivation of the responding cells during the cell fractionation procedure.

Effect of mitomycin C treatment on the mitogenic response

MNL were fractionated into three cell fractions by the combination of the plastic adherence method and the E-rosette sedimentation method, as described in Fig.2. The separated cell fractions were treated with mmc. As shown in Table VI, the proliferative response of the mmc-treated E-R(+) cell fraction to Con.A, PWM and sample H 52-2 was markedly reduced compared to the response of an untreated culture. In the mixture of the E-R(+) and adherent cell fractions, when the adherent cell fraction had been pretreated with mmc, a high proliferative response to PWM and sample H 52-2 was observed, whereas, when the E-R(+) cell fraction was pretreated with mmc, the response was markedly reduced, especially in the case of sample H 52-2.

FIG. 1. Effect of Combining T-Enriched Cells with T-Depleted Cells on the Mitogen Response.

The final cell concentration of the mixed culture of T-enriched and T-depleted cells was 5.0×10^5 cells per ml. Incorporation of [³H]-thymidine into the culture was measured in the presence of sample H 52-2 (■, 5 μ l) and in its absence (●). Each value is the square root of the product of the cpm values in two experiments (two MNL donors). UF denotes the culture of unfractionated cells.

FIG. 2. Fractionation Scheme for Human Blood Mononuclear Leukocytes.

E-R(+) and E-R(-) cells refer to rosette formation with SRBC positive and negative cells, respectively.

On the other hand, in the mixed culture of the E-R(+) and mmc-treated E-R(-) cell fractions and that of the mmc-treated E-R(+) and E-R(-) cell fractions, the proliferative re-

TABLE VI. EFFECT OF MITOMYCIN C TREATMENT OF HUMAN MNL SUBSETS ON THE MITOGEN RESPONSE

Each cell fraction of separated MNL subsets consisted of 2.5×10^5 cells per ml except for unfractionated MNL, which consisted of 5×10^5 cells per ml. Each value represents the radioactivity incorporated into cells.

	Con. A (25 μ g/ml)	PWM (1 μ g/ml)	H 52-2 (5 μ l)	Control ^c
Unfractionated MNL	36,400	13,000	7,760	1,020
E-R(+)	30,500	7,920	728	174
E-R(+) _{mmc} ^b	5,420	827	85	80
E-R(+)+A _{mmc}	40,100	11,600	5,960	175
E-R(+) _{mmc} +A ^a	15,000	7,830	1,850	381
E-R(+)+E-R(-) _{mmc}	30,800	7,860	4,510	197
E-R(+) _{mmc} +E-R(-)	14,000	5,330	5,180	417

^a Adherent cell fraction.

^b Treated with mitomycin C.

^c Values in the absence of mitogens.

sponse to PWM and sample H 52-2 was about a half of that in the case of unfractionated MNL.

DISCUSSION

From the results of our screening, it is concluded that marine bacteria, especially marine vibrios, are rich sources of mitogenic substances for human lymphocytes. Oishi *et al.* systematically screened hemagglutinating activity in microorganisms and showed that highly active strains were present in marine vibrios.¹³⁾ Mitogen samples from our selected strains did not agglutinate mammalian erythrocytes, in which they differ from bacterial lectins. They also differ from plant mitogenic lectins. They are human specific, whereas plant mitogens display broad species specificity.

Human lymphocyte mitogens of microbial origin were known to be distributed predominantly in pathogenic strains as toxic substances or to be components of the cell wall or cell membrane; *i.e.*, pyrogenic exotoxin,¹⁴⁾ M protein¹⁵⁾ and lipoteichoic acid¹⁶⁾ of *Streptococcus pyogenes*, pyrogenic exotoxin,¹⁷⁾ enterotoxin B,¹⁸⁾ toxic shock toxin,¹⁹⁾ protein A,²⁰⁾ Cowan I,²¹⁾ peptidoglycan,²²⁾ teichoic acid²³⁾ and cytoplasmic membrane²⁴⁾ of *Staphylococcus aureus*, water soluble mitogen²⁵⁾ of *Nocardia opaca*, polysaccha-

ride²⁶⁾ of *Candida albicans*, protein fraction of cell wall²⁷⁾ of *Salmonella typhimurium*, extracellular slime layer²⁸⁾ and galactosephilic lectin²⁹⁾ of *Pseudomonas aeruginosa* and culture broth³⁰⁾ of *Mycoplasma arthritidis*. Rare exceptions are mycelia³¹⁾ and bestatin³²⁾ of nonpathogenic *Streptomyces*. The present human lymphocyte mitogens were not toxic to mice, so far as examined at the level of 5 ml i.p. injection per mouse, and obtained from marine bacteria which do not shown human or fish pathogenicity.

The present mitogens have not yet been purified from the culture filtrate. However, it is considered to be a low molecular weight substance because most of the activity passed through a cellulose tube and hollow fiber HI (Asahi Chemical Industry, Co., Tokyo), and it was found to have a M_r in the range of 1000~1300 on Sephadex G-50 column chromatography. It could not be extracted from the culture filtrate with *n*-butanol, ethyl acetate or benzene at pH 3, 5 or 12, and hardly adsorbed to DEAE Sephadex in the pH range of 5.0~9.0. Heating at 60°C for 10 min caused a little decrease in the mitogenic activity whereas that at 80°C resulted in a 55% decrease. The remaining activity was quite stable and heating at 100°C had no further effect on the activity.

With the exception of bestatin, the microbial

mitogens mentioned above have high molecular weights and are heat labile. In these points, the present mitogen is considered to be a novel one.

The mitogen response of human lymphocytes to plant mitogens requires cell-cell interaction; *i.e.*, the proliferative response of human T cells to Con.A only occurs in the presence of monocytes,³³⁾ and the stimulation by PWM of human B cells to proliferate and differentiate into immunoglobulin secreting cells requires both T cells³⁴⁾ and monocytes.³⁵⁾ As to cell-cell interaction in the mitogen response to bacterial sample H 52-2, it is not obvious that macrophages and monocytes play crucial roles, because depletion of adherent cells from a human MNL culture resulted in only a little decrease in the response. However, a small number of macrophages and monocytes contaminated in the nonadherent cell preparation may fully help the proliferative response of the lymphocytes. The present results indicate that T cells play a major role in the mitogen response, and the full response requires cell-cell interaction between T cells and other cells. The results of the experiment involving mmc treatment indicate that the cells proliferating in response to sample H-52-2 form 2 classes; T cells and non-T lymphocytes. In the former case, non-T lymphocytes or macrophages (monocytes) play an accessory role in proliferation of T cells, and in the latter case, T cells play an accessory role in the proliferation of non-T lymphocytes.

REFERENCES

- 1) N. Sharon, *Adv. Immunol.*, **34**, 213 (1983).
- 2) I. Gery, J. Kruger and S. Z. Spiesel, *J. Immunol.*, **108**, 1088 (1972).
- 3) A. S. Kong and S. I. Morse, *J. Exp. Med.*, **145**, 151 (1977).
- 4) K. Ariizumi, K. Oishi and K. Aida, *J. Gen. Appl. Microbiol.*, **26**, 239 (1980).
- 5) A. Böyum, *Scan. J. Clin. Lab. Invest.*, **21** (Suppl., **97**), 1 (1968).
- 6) R. Dulbecco, *J. Exp. Med.*, **99**, 169 (1954).
- 7) G. Janossy and M. F. Greaves, *Clin. Exp. Immunol.*, **9**, 483 (1971).
- 8) M. Jondal, G. Holm and H. Wigzell, *J. Exp. Med.*, **136**, 207 (1972).
- 9) U. Galili and M. Schlesinger, *J. Immunol.*, **112**, 1628 (1974).
- 10) R. Evans, *J. Reticuloendocrinol. Soc.*, **8**, 571 (1970).
- 11) S. T. Cowan, "Manual for the Identification of Medical Bacteria," 2nd Ed., Cambridge Univ. Press, 1974.
- 12) E. R. Unanue, *Nature*, **218**, 36 (1968).
- 13) K. Oishi, S. Yokoshima, T. Tomiyama and K. Aida, *Appl. Environ. Microbiol.*, **38**, 169 (1979).
- 14) P. M. Schlievert, D. J. Schoettle and D. W. Watson, *Infect. Immun.*, **27**, 276 (1980).
- 15) H. Knöll, O. Kühnemund and J. Havlicek, *Immunobiol.*, **156**, 537 (1979).
- 16) E. H. Beachey, J. B. Dale, S. Grebe, A. Ahmed, W. A. Simpson and I. Ofek, *J. Immunol.*, **122**, 189 (1979).
- 17) P. M. Schlievert, D. J. Schoettle and D. W. Watson, *Infect. Immun.*, **25**, 1075 (1979).
- 18) D. L. Peavy, W. H. Adler and R. T. Smith, *J. Immunol.*, **105**, 1453 (1970).
- 19) S. E. Calvano, F. W. Quimby, A. C. Anthony, R. F. Reiser, M. S. Bergdoll and P. Dineen, *Clin. Immunol. Immunopathol.*, **33**, 99 (1984).
- 20) A. Forsgren, A. Svedjelund and H. Wigzell, *Eur. J. Immunol.*, **6**, 207 (1976).
- 21) J. Pryjma, J. Munoz, R. M. Galbraith, H. H. Fudenberg and G. Virella, *J. Immunol.*, **124**, 656 (1980).
- 22) R. Dziarski and A. Dziarski, *Infect. Immun.*, **23**, 706 (1979).
- 23) R. Dziarski, A. Dziarski and A. I. Levinson, *Int. Archs. Allergy Appl. Immunol.*, **63**, 383 (1980).
- 24) H. Takada, Y. Hirachi, H. Hashizume and S. Kotani, *Microbiol. Immunol.*, **25**, 317 (1981).
- 25) J. Brochier, C. Bona, R. Ciorbaru, J. P. Revillard and L. Chedid, *J. Immunol.*, **117**, 1434 (1976).
- 26) E. Piccolella, G. Lombardi and R. Morelli, *J. Immunol.*, **125**, 2082 (1980).
- 27) M. B. McChesney, C. J. Froelich, J. L. Cantrell and R. C. Williams, Jr., *Cell. Immunol.*, **93**, 83 (1985).
- 28) M. Papamichail, G. Dimitracopoulos, G. Tsokos and J. Papavassiliou, *J. Infect. Dis.*, **141**, 686 (1980).
- 29) Y. Sharabi and N. Gilboa-Garber, *FEMS Microbiol. Lett.*, **5**, 273 (1979).
- 30) R. A. Daynes, J. M. Novak and B. C. Cole, *J. Immunol.*, **129**, 936 (1982).
- 31) B. F. Maier, D. P. Baron, H. Bockhorn, H. Drautz, J. Langharig and W. G. Bessler, *Immunobiol.*, **163**, 427 (1982).
- 32) M. Ishizuka, J. Sato, Y. Sugiyama, T. Takeuchi and H. Umezawa, *J. Antibiot.*, **33**, 653 (1980).
- 33) R. Palacios, *Immunol. Rev.*, **63**, 72 (1982).
- 34) T. Hirano, T. Kuritani, T. Kishimoto and Y. Yamamura, *J. Immunol.*, **119**, 1235 (1977).
- 35) S. A. Rosenberg and P. E. Lipsky, *J. Immunol.*, **122**, 926 (1979).