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ANTIPROLIFERATIVE AND IMMUNOSUPPRESSIVE PROPERTIES OF MICROCOLIN A, A MARINE-DERIVED LIPOPEPTIDE

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Summary

The immunosuppressive effects of microcolin A, a lipopeptide extracted from the marine blue green alga *Lyngbya majuscula* were investigated. Microcolin A suppressed concanavalin A (IC_{50} = 5.8 nM), phytohemagglutinin (IC_{50} = 12.5 nM) and lipopolysaccharide (IC_{50} = 8.0 nM) induced proliferation of murine splenocytes. Mixed lymphocyte reaction (IC_{50} = 5.0 nM), anti-IgM (μ-chain specific) (IC_{50} = 10.0 nM), and phorbol 12-myristate 13-acetate plus ionomycin (IC_{50} = 5.8 nM) stimulation of murine splenocytes were all similarly suppressed by microcolin A. The inhibitory activity of microcolin A was time-dependent and reversible and was not associated with a reduction in cell viability. Moreover, microcolin A not only inhibited IL-2 production and IL-2 receptor expression by concanavalin A activated splenocytes, but also suppressed *in vitro* antibody responsiveness to keyhole limpet hemocyanin. These results indicate that microcolin A is a potent immunosuppressive and antiproliferative agent.

Key Words: microcolin A, lymphocyte proliferation, immunosuppressant

The present success of organ and bone marrow transplantation for the treatment of immunodeficiency diseases and cancer is directly attributable to the discovery of new classes of chemical compounds obtained from the systematic screening of extracts obtained from terrestrial plants and animals. These efforts have yielded novel chemical compounds, which either have directly or through structural modification become clinically useful immunotherapeutic agents. Cyclosporin A, a cyclic undecapeptide derived from the fungus *Tolypocladium inflatum*, has continued to show remarkable success over the past 15 years in both organ and bone marrow transplantation (1, 2, 3). The macrolides FK-506 (tacrolimus) and the structurally related rapamycin (sirolimus) are additional examples of potent immunosuppressive agents derived from Streptomycetes. FK-506 has demonstrated a successful record of clinical utility in liver, kidney and pancreas transplantation and has recently become openly available for use after organ transplantation (tradename Prograf), while rapamycin has shown similar promise in preclinical studies in rodents (4, 5).

Our group has focused its research on the discovery of marine natural products which exhibit potent immunoregulatory activity as mediated both directly and indirectly through interaction with various cells of the immune system. As a result, immunosuppressive sterols and pyrrole metabolites were discovered from the deep-water sponge *Agelas flabelliformis* (6), and a novel polyhydroxylated alkatetraene lactone, discodermolide, was isolated from the Caribbean sponge

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Discodermia dissoluta and characterized as a potent immunosuppressive and antiproliferative agent. This compound was found to induce apoptosis in various mouse and human tumor cells and to stabilize the assembly of microtubules more potently than taxol (7, 8, 9, 10, 11).

Recently, our ongoing interest in the isolation and screening of immunomodulatory peptide secondary metabolites from marine organisms led us to find two novel lipopeptides, microcolin A and B, which were isolated from the marine blue green alga *Lyngbya majuscula* (12). Microcolin A (Fig. 1) proved primarily to be a more potent inhibitor of murine mixed lymphocyte response and murine P-388 leukemia *in vitro* with relatively less toxicty than microcolin B (13). In this paper, we have investigated antiproliferative and immunosuppressive effects of microcolin A in detail. The comparative study of this compound with its analogs obtained by semisynthetic modification and chemical degradation will be reported in a subsequent paper.

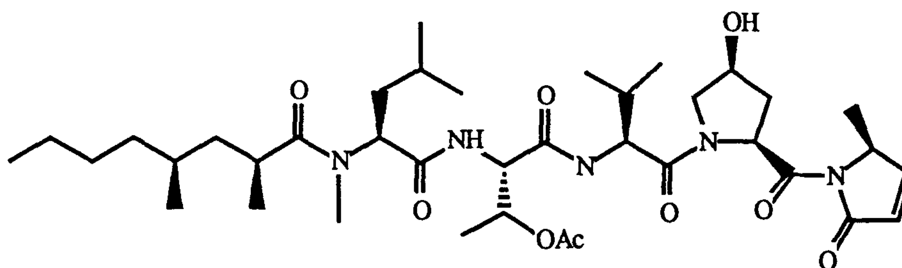

Fig. 1

Chemical structure of microcolin A (Mic A, $C_{39}H_{65}O_9N_5$, mol. wt. 747)
(U.S. Patent No. 5091368 and European Patent No. 0542891)

Materials and Methods

Reagents

RPMI-1640 medium with *l*-glutamine, fetal calf serum (FCS), and non-essential amino acids were obtained from Gibco Co. (Grand Island, NY). Phytohemagglutinin (PHA), concanavalin A (Con A), lipopolysaccharide (LPS), phorbol 12-myristate 13-acetate (PMA), ionomycin (ION), affinity purified goat-anti-mouse IgM (μ-chain specific), 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), keyhole limpet hemocyanin (KLH), anti-mouse IgG (whole molecule) alkaline phosphate conjugate, and *p* -nitrophenyl phosphate were purchased from Sigma Co. (St. Louis, MO). IL-2 ELISA kits were purchased from Endogen Co. (Cambridge, MA). FITC conjugated rat anti-mouse CD_{25} (IL-2 receptor, α chain) monoclonal antibody (MAb) were purchased from PharMingen Co. (San Diego, CA).

Drugs

Microcolin A (MicA), mol. wt. 747, was isolated from frozen samples of *Lyngbya majuscula* according to our previously published method (12). Cyclosporin A (Sandimmune) was kindly provided by Sandoz Research Institute (East Hanover, NJ). Stock solutions (1mg/ml) of both drugs were prepared with absolute ethanol and diluted with tissue culture medium (TCM) to give the required test concentration. The concentration of ethanol in the vehicle control was 1/20,000 (v/v), the highest concentration in MicA groups.

Mice

Pathogen-free, 6-8 week-old male Balb/c (H-2^d) and C_{57}BL/6 (H-2^b) inbred mice were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN). They were housed in sterile cages with sterile bedding in well-ventilated rooms at room temperature and fed on a standard diet and sterilized water *ad libitum.*

Preparation of murine splenocytes

Splenocyte suspensions were prepared as described elsewhere (7). Briefly, mice were sacrificed by cervical dislocation, spleens excised, and single cell suspensions prepared by grinding the spleen with the plastic end of a sterile plunger of a 10 ml syringe in 10 ml of cold 10% FCS RPMI-1640 TCM. Debris was removed with a 70 µm Falcon cell strainer (Becton-Dickinson, NY) and the cells were recovered by centrifugation at 300 *g*. The erythrocytes were removed by osmotic lysis using Tris-buffered ammonium chloride, washed twice and resuspended with TCM. Cell viability was determined by trypan blue dye exclusion and the suspensions were adjusted to give the required number of viable spleen cells/ml.

Proliferative response of murine splenocytes

Lymphocyte proliferative responses were tested as previously described (14). Briefly, a total 4 x10^5 splenocytes/well from Balb/c mice were added in triplicate to flat-bottomed microtiter plates, to which 2 µg/ml ConA, 20 µg/ml PHA, 10 µg/ml LPS, 3.125 ng/ml PMA plus 0.313 µM ION, or 20 µg/ml goat-anti-mouse IgM (µ-chain specific) was added. The plates were incubated with various concentrations of MicA, in a final volume of 200 µl of TCM. Following incubation at 37°C in a 5% CO_2 humidified atmosphere for 66 h, 150 µg MTT in 75 µl PBS was added to each well and the plates were incubated for an additional 4 h. After this period, cell supernatants were discarded and 200 µl/well of acidified (0.04N HCl) isopropanol was added to dissolve the formazan crystals. The optical density ($OD_{570/630nm}$) was measured on an ELISA reader using a test wavelength of 570 nm and a reference wavelength of 630 nm. The median inhibition concentration (IC_{50}) was defined as the drug concentration required to inhibit proliferative responses of lymphocytes by 50%, and calculated from linear regression analysis of plotted values.

Two-way mixed lymphocyte reaction

Splenocytes from Balb/c (H-2^d) and C_{57}BL/6 (H-2^b) were prepared as described above, and a volume of 100 µl of each cell suspension (5x10^6 cells/ml) was added together to test and control wells of microtiter plates. Wells containing 200 µl of each cell suspension alone also served as controls. Serial ten-fold dilutions of MicA were added directly to the wells containing cell combinations. Plates were incubated at 37°C in a 5% CO_2 humidified atmosphere for 90 h. Following the incubation, 150 µg MTT in 75 µl PBS was added to each well and the incubation was continued for an additional 4 h prior to determination of $OD_{570/630nm}$.

IL-2 production and determination

Murine splenocytes (2.5x10^6) were incubated with 4 µg/ml ConA, with or without the indicated concentrations of Mic A at 37°C for 24 h. Culture supernatants were harvested and IL-2 levels were determined by an indirect enzyme-linked immunosorbent assay (ELISA) using a protocol supplied with the kits from Endogen Co. with minor modification. In brief, 96-well flat-bottomed microtiter plates were coated with anti-mouse IL-2 MAb in PBS at room temperature overnight. The plates were then blocked with 2% bovine serum albumin in PBS containing 0.01% thimerosal at 37°C for 1 h. After being washed with wash buffer (50 mM Tris, 0.2% Tween-20, pH 7.2), the plates were incubated with recombinant mouse IL-2 standard or samples at room temperature overnight. The plates were washed and then treated with biotinylated detecting antibody. After washing, the plates were treated with a 1:2000 dilution of horseradish peroxide (HRP)-conjugated streptavidin at 37°C for 30 min. After final washing, HRP-streptavidin was detected by addition of

a tetramethylbenzidine substrate. Color development was stopped with 0.18 M H_2SO_4 and OD_{405nm} was measured with a microplate reader.

IL-2 receptor expression

The presence of membrane associated IL-2 receptors was determined using FITC conjugated rat anti-mouse CD_{25} MAb. Splenocytes from the IL-2 production cultures described above were incubated at 4°C for 50 min with 1: 50 dilution of CD_{25} MAb, then cells were washed three times with cold PBS. The resulting cell suspensions were analyzed using an EPICS Elite Flow Cytometer (Coulter Co., Hialeah, FL).

Induction of anti-KLH antibody

Balb/c mice were immunized intraperitoneally with 1 mg KLH in 100 µl PBS. Splenocytes were prepared after 4 weeks, plated at 2.5×10^6 cells/well and stimulated with 7 µg/ml KLH, in the presence of various concentrations of MicA or medium alone, in a final volume of 250 µl in 96-well flat-bottomed microtiter plates. After 6 days incubation, the cells were washed three times with TCM, and resuspended in 300 µl TCM. The cell suspensions were incubated for 24 h and the supernatants collected for analyses.

Detection of anti-KLH antibody

The presence of anti-KLH antibody in spleen cell supernatants was determined by ELISA. Round-bottomed, 96-well culture plates were coated with KLH (10 µg/ml) in carbonate / bicarbonate buffer (pH 9.6) for 3 h at 37°C. The plates were washed with 0.05% Tween-20 in PBS and test samples added at 150 µl/well. Following a 2 h incubation at 37°C, the plates were washed four times, and 150 µl of a 1: 2500 dilution of rabbit anti-mouse IgG (whole molecule) alkaline phosphatase-conjugate was added. The plates were then incubated overnight at 4°C and washed four times before adding 150 µl/well of *p*-nitrophenyl-phosphate substrate at 1 mg/ml in carbonate buffer. Color development was stopped after 30 min incubation at room temperature by the addition of 25 µl 1 M NaOH. Absorbance was measured at 405 nm.

Cytotoxicity evalution on mouse splenocytes

Unstimulated mouse splenocytes were utilized to determine the direct cytotoxic activity of MicA for lymphocytes. Cells (1×10^5) were inoculated in 96-well flat-bottomed microtiter plates in a total volume of 200 µl of TCM. MicA at the indicated concentrations was added at the beginning of the culture. After incubation for 24, 48, or 72 h, cell viablity was determined by trypan blue exclusion method.

Results

Antiproliferative effects of MicA on mouse splenocytes

The effects of MicA on mouse splenocyte proliferation induced by the T-cell mitogens ConA and PHA, and allotypic antigen in the mixed lymphocyte reaction (MLR) were investigated. The results in Fig. 2 show that MicA suppressed proliferation of activated splenocytes in a dose-dependent fashion. Half-maximal inhibition of lymphocyte proliferation was apparent with MicA at concen-trations of 5.8 nM, 12.5 nM and 5.0 nM for ConA, PHA and MLR, respectively.

The effects of MicA on mouse splenocyte proliferation induced by the non-specific B cell mitogen LPS, the T-helper independent B-cell mitogen anti-mouse IgM (µ-chain specific) and a combination of a phorbol ester PMA and a calcium ionophore ION were also determined. Data presented in Fig. 3 shows that MicA inhibited the proliferation of activated splenocytes in a dose-dependent fashion. Splenocyte proliferation induced by LPS, anti-IgM or PMA plus ION was reduced to a

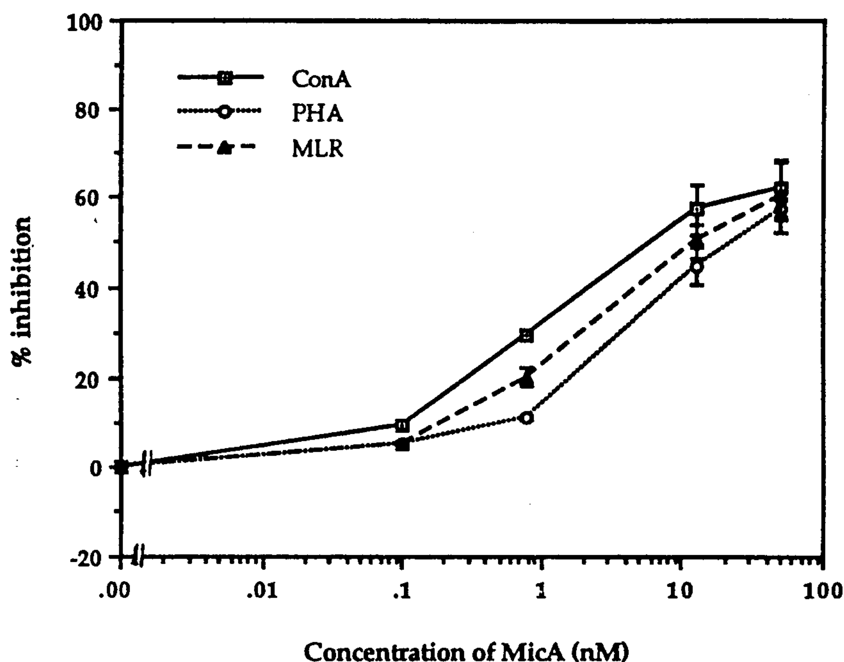

Fig. 2
Inhibitory effect of MicA on murine splenocyte proliferation. Splenocytes from Balb/c mice were stimulated with ConA (2 μg/ml), PHA (20 μg/ml), or with allogenic splenocytes from $C_{57}BL/6$ mice in the presence or absence of various concentrations of MicA. Data are the mean percentage inhibition with S.D. of three experiments.

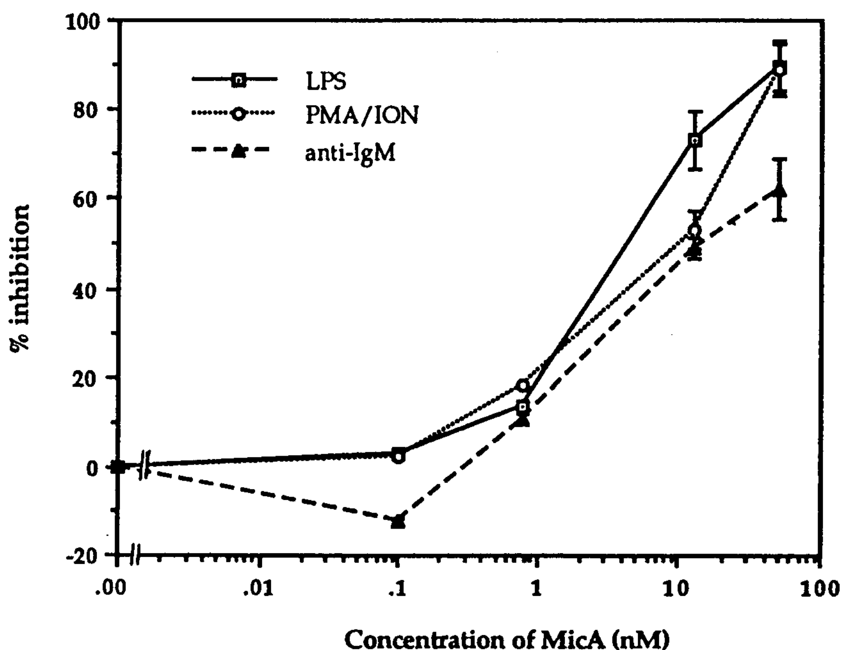

Fig. 3
Inhibitory effect of MicA on murine splenocyte proliferation. Splenocytes from Balb/c mice were stimulated with LPS (10 μg/ml), goat anti-mouse IgM (20 μg/ml) or PMA (3.12 ng/ml) plus ION (0.313μM) in the presence or absence of various concentrations of MicA. Data are the mean percentage inhibition with S.D. of three experiments.

half-maximal level by MicA at concentrations of 8.0 nM, 10.0 nM and 12.5 nM, respectively. These results indicate that MicA suppresses the response of both T- and B-cells.

Moreover, splenocytes in the above experiments exposed in the presence of test concentrations of MicA appeared normal when viewed microscopically and were viable as determined by their ability to exclude trypan blue (data not shown).

Comparison of the antiproliferative effects of MicA and cyclosporin A

The immunosuppressant cyclosporin A was evaluated as a reference standard in the splenocyte proliferation assays described above. Data presented in Table 1 show the concentrations of cyclosporin A which caused a half-maximal inhibition of lymphocyte proliferation along with the equivalent values of MicA for comparison. Cyclosporin A and MicA both inhibited lymphocyte proliferation induced by these stimuli, but lymphocyte proliferations were inhibited by MicA at a lower concentration as compared with cyclosporin A.

TABLE 1

Comparison of the median concentrations of cyclosporin A and microcolin A for inhibition of murine splenocyte proliferation

Stimulator	IC_{50} value (nM)	
	Microcolin A	Cyclosporin A
ConA	5.8	68.4
LPS	8.0	480.0
PHA	12.5	15.5
MLR	5.0	35.8
anti-IgM	10.0	18.8
PMA+ION	12.5	64.2

Kinetics of antiproliferative effects of MicA on mouse splenocytes

In all of the above experiments MicA at various concentrations was added at the beginning of cell activation and remained in culture during the entire incubation time (3 to 4 days). In the following kinetic experiments MicA (50 nM) was added at designated time points following the start of cell activation. The results in Fig. 4 show that the inhibitory effect of MicA on lymphocyte proliferation was dependent on the time that the drug was added to the cultures: MicA was effective when added within the first 3 h after the onset of ConA-induced splenocyte proliferation or when added only within the first 6 h after the onset of PMA plus ION induced proliferation. Fig. 5 shows that MicA suppressed LPS induced splenocyte proliferation of B-cells when added within 36 h after initiation of culture. Anti-IgM induced splenocyte proliferation was suppressed by MicA only when added at the beginning of cell activation or between 12-54 h after starting of the cultures. These results indicate that the kinetics of the antiproliferative/ immunosuppressive properties of MicA varied according to both the lymphocyte subpopulation and induction stimulus.

Reversibility of antiproliferative effects of MicA on mouse splenocytes

The results in Table 2 show that splenocytes which were activated with a combination of PMA and ION and were treated with MicA (50nM), were suppressed in their ability to proliferate. However, PMA plus ION stimulated splenocytes which were washed free of MicA following 24 h incubation demonstrated no appreciable decrease in OD[illegible] values compared with that of control

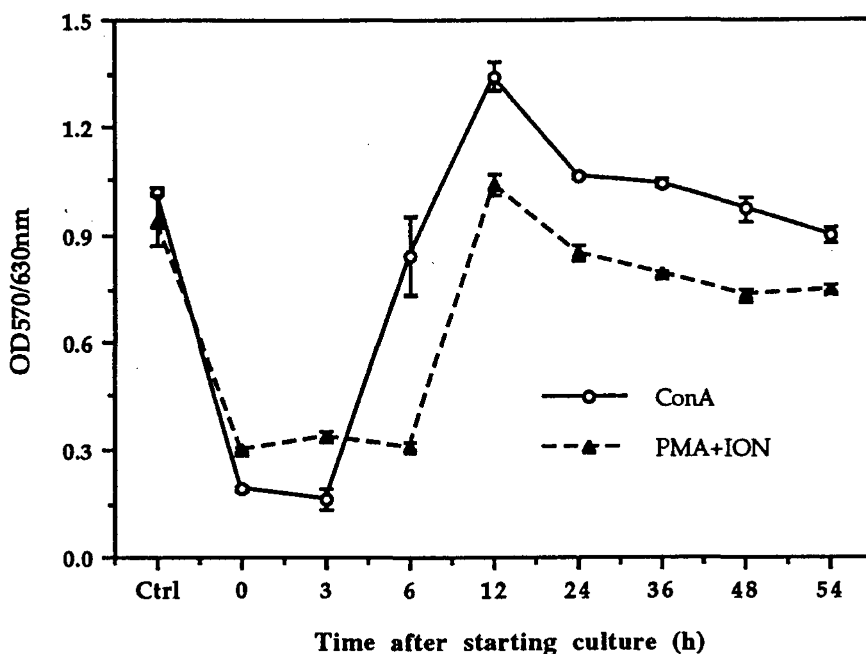

Fig. 4
Time kinetics of the antiproliferative effect of MicA (50 nM) on mouse splenocytes. Splenocytes were stimulated with ConA (2 μg/ml) or PMA (3.12 ng/ml) plus ION (0.313 μM) for 66 h. Data are the mean±S.D. from one experiment out of three similar ones with triplicate cell incubations. Ctrl, control incubation in the absence of MicA.

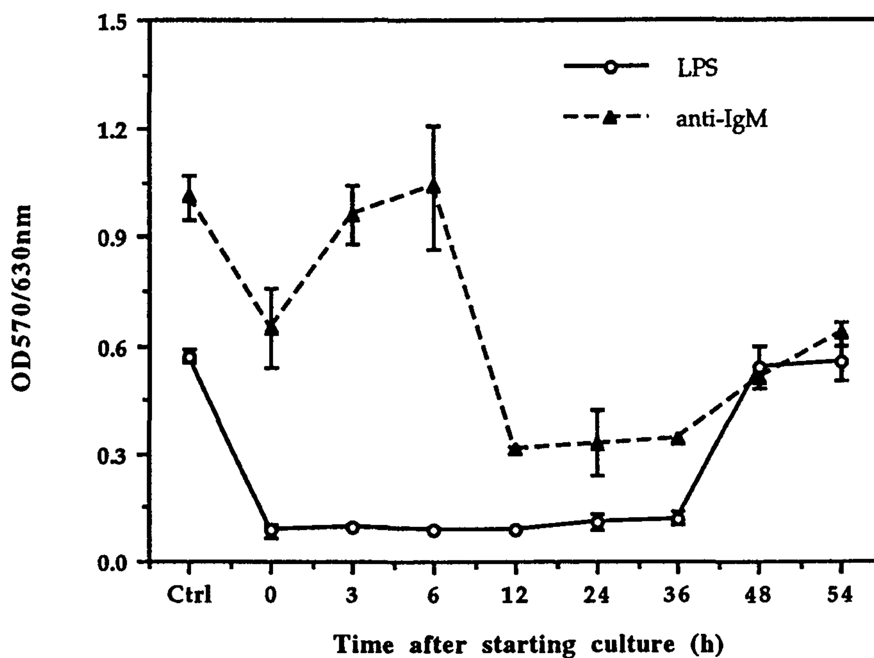

Fig. 5
Time kinetics of the antiproliferative effect of MicA (50 nM) on mouse splenocytes. Splenocytes were stimulated with LPS (10 μg/ml) or goat anti-mouse IgM (20 μg/ml) for 66 h and proliferation determined by MTT method. Data are the mean±S.D. from one experiment out of three similar ones with triplicate cell incubations. Ctrl, control incubation in the absence of MicA.

cultures. Subsequent addition of MicA after washing resulted in a substantial inhibition of cell proliferation, indicating that splenocyte populations which remained were still capable of being suppressed in their proliferation by MicA.

TABLE 2

Reversibility of antiproliferative effects of microcolin A on mouse splenocytes activated with PMA plus ION

Groups	$OD_{570/630nm}$	% of control
Control	1.15±0.07	
MicA 50 nM		
(present for 72 h)	0.37±0.04	32.2
(washed out after 24 h)	1.27±0.10	108.5
(washed out after 24 h and re-added for 48 h)	0.18±0.02	15.6

Proliferation of mouse splenocytes stimulated with PMA plus ION for 3 days in the presence or absence of MicA at a fixed concentration (50 nM) was determined by MTT method.MicA was either present for the entire incubation time or washed out after 1 day and then the culture was continued for 2 days in the absence or after re-addition of MicA (50 nM). Data are mean±S.D. from triplicate cell incubations of one experiment representative of three.

Inhibitory effect of MicA on IL-2 production and IL-2 receptor expression by mouse splenocytes

To determine whether the MicA-induced suppression of splenocyte proliferation was related to reduced IL-2 production, we evaluated the IL-2 content in the supernatants of MicA-treated cultures. Fig.6 shows that MicA markedly suppressed IL-2 production of ConA-activated splenocytes in a dose-dependent fashion. Staining of these MicA treated splenocytes with FITC labelled anti-CD25 antibody revealed a significantly reduced number of $CD25^+$ cells as compared with untreated controls (Fig. 7). These results indicate that MicA reduced both the amount of IL-2 production as well as the number of IL-2 receptor bearing cells in ConA-stimulated splenocytes.

Inihibitory effect of MicA on antibody production

The effect of MicA on the production of anti-KLH antibody by splenocytes harvested from KLH-primed mice that were stimulated with KLH *in vitro* was also investigated. Fig. 8 shows that MicA suppressed the production of anti-KLH antibody in a concentration responsive fashion. MicA did not appreciably affect the viability of splenocytes as described above.

Effect of MicA on cell viability of mouse splenocytes

Mouse splenocytes were continuously exposed to various concentrations of MicA for 24, 48, or 72 h, and the percentage of viable cells determined using trypan blue exclusion. The results in Fig 9 showed that no significant decrease in the percentage of viable cells compared with control cells was observed when cells were exposed to MicA (0 - 100 nM) for 24 or 48 h. MicA (> 100 nM) was found to show a substantial toxicity to cells after 72 h of incubation.

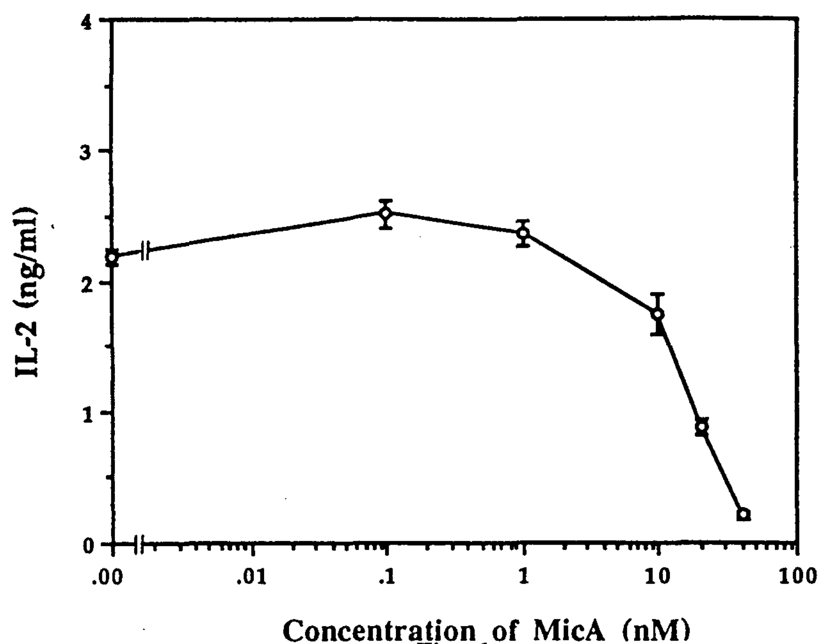

Fig. 6

Inhibitory effect of MicA on IL-2 production by mouse splenocytes. Splenocytes from Balb/c mice were stimulated by ConA (2 μg/ml) in the presence or absence of various concentrations of MicA. Culture supernatants were removed after 24 h incubation and IL-2 level quantified by ELISA. Data are the mean± S.D. of three experiments.

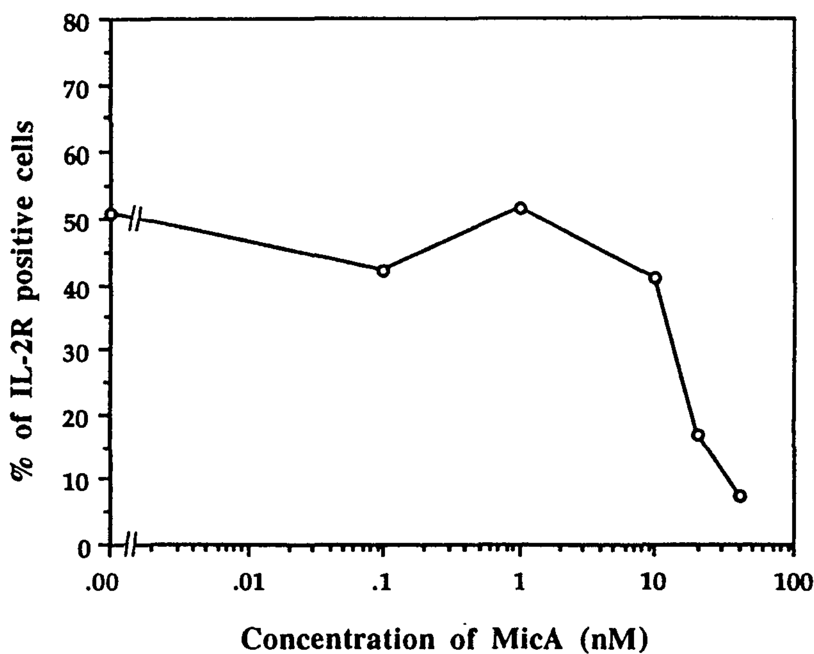

Fig. 7

Effect of MicA on IL-2 receptor expression of mouse splenocytes. Splenocytes from Balb/c mice were stimulated with ConA (2 μg/ml) in the presence or absence of various concentrations of MicA for 24 h at 37°C. The presence of IL-2 receptors was detected using FITC-labeled rat anti-mouse CD_{25} MAb. Results are from one experiment representative of three.

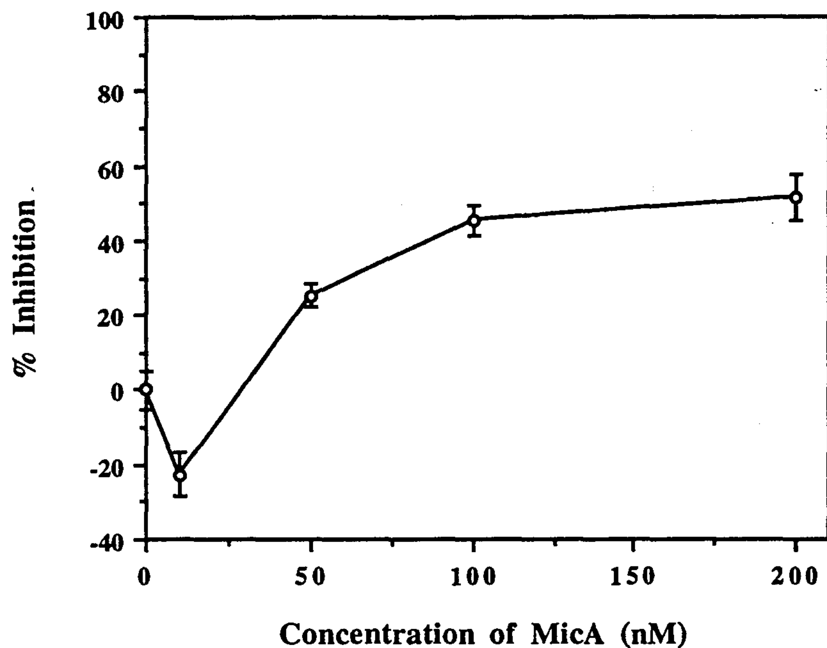

Fig. 8
Inhibitory effect of MicA on anti-KLH antibody production *in vitro*. Splenocytes were harvested from KLH-primed Balb/c mice and stimulated with KLH (7 µg/ml) *in vitro* in the presence or absence of MicA. Cells were washed after 6 days' incubation and supernatants removed after a further 24 h culture for detection of anti-KLH antibody by ELISA.

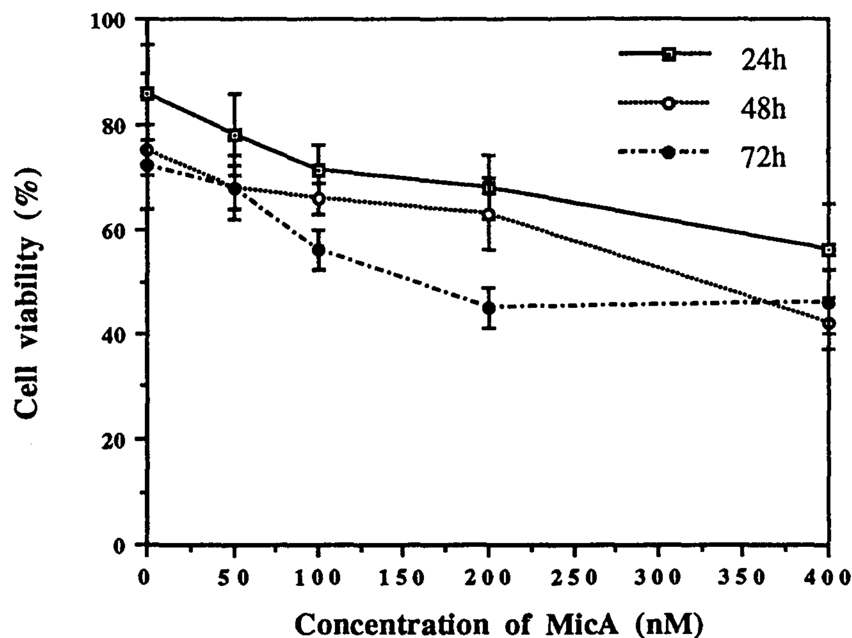

Fig. 9
Effect of MicA on viability of mouse splenocytes. Data are the mean±S.D. from one experiment out of two similar ones with triplicate cell incubations. Cell viabilty was determined by trypan blue exclusion method after cells were incubated for 24, 48, or 72 h in the presence or absence of various concentrations of MicA.

Discussion

In the course of our screening efforts to discover novel, less-toxic immunotherapeutic agents derived from marine organisms, several immunosuppressive lipopeptide compounds were isolated from a sample of the blue-green alga *Lyngbya majuscula*. A preliminary analysis of the immunological effects of these compounds led us to further study microcolin A (MicA), which possessed a higher therapeutic index as an inhibitor of the murine mixed lymphocyte response (12,13). The present studies were undertaken to determine the effect of MicA on a variety of immune reactions *in vitro*.

Our data shows that MicA suppressed T-cell proliferation in mouse splenocytes induced by the T-cell mitogens ConA, PHA and by allotypic antigen in the mixed lymphocyte reaction. MicA also suppressed the proliferation of B-cells in splenocytes induced by the B-cell mitogens anti-IgM or LPS. The compound also blocked the production of anti-KLH antibody from KLH-primed and stimulated splenocytes *in vitro*. Previous studies (15) have shown that LPS and anti-IgM antibodies trigger B-cell proliferation via different activation pathways. The cross-linking of surface immunoglobulin receptors on B-cells by anti-IgM antibodies induces phosphatidylinositol bisphosphate degradation to diacylglycerol and inositol triphosphate. These activate protein kinase C (PKC) and cause calcium mobilization in B-cells. LPS does not induce either of these responses in resting B-cells (16). Therefore, our observations suggest that MicA may affect more than one activation pathway in B-cells, or block a downstream event which leads to proliferation. MicA may inhibit antibody production by blocking B-cell activation and/or inhibiting IL-2 release by T-helper cells which promote the activation and differentiation of B-cells into antibody secreting plasma cells. Subsequent results confirmed the IL-2 production and IL-2 receptor expression of ConA-activated splenocytes was markedly suppressed by MicA.

Our studies also show that the proliferation of both T-cells and B-cells induced by a variety of stimuli was significantly suppressed by MicA, but it remains to be clarified whether MicA suppresses the proliferative response of B-cells and T-cells by the same molecular mechanism. However, our data does suggest that MicA may affect lymphocyte activation at a stage which closely follows an increase in the intracellular Ca^{2+} concentration and the activation of PKC. This hypothesis is based on the demonstration that MicA suppressed lymphocyte proliferation induced *in vitro* with a combination of the phorbol ester PMA and the calcium ionophore ION.

In time kinetic experiments, we have obtained evidence that the antiproliferative effect of MicA is based on the impairment of early steps which occur during lymphocyte activation. MicA was effective only when added to cultures in the first 3-6 h after the onset of splenocyte proliferation stimulated by ConA or by PMA plus ION. After prolonged incubation times, the addition and then continuous presence of MicA was without an appreciable effect. MicA also suppresses B-cell proliferation when added during the first 36 h to cultures of splenocytes stimulated with LPS, and also when added at the same time as or within the period of 12-54 h after the beginning of culture of splenocytes which were activated by anti-IgM. The different kinetic features displayed by MicA may be the result of different mechanisms by which these agents stimulate lymphocyte proliferation. From these results it can also be concluded that MicA is not simply cytotoxic for lymphocytes. Accordingly, we demonstrated that MicA acts in a reversible manner. When the agent was incubated with splenocytes which had been stimulated with PMA plus ION for 24 h and then removed by washing, the cells regained their ability to proliferate.

The antiproliferative action of MicA was not associated with a reduction in viability. Microscopic observation of splenocytes continually exposed to MicA at the various test concentrations revealed the presence of intact, trypan blue dye excluding cells that were determined to be viable. In addition, the percentage of intact cells in thymocyte cultures incubated with MicA overnight was unchanged as determined by fluorescein labelled annexin-V binding in conjugation with propidium iodide exclusion method (17), though the percentage of early apoptotic cells was significantly increased (Zhang, et al., in preparation).

It is well known that IL-2 plays a pivotal role in T-cell proliferation. The widely used immunosuppressants, cyclosporin A and FK-506, are reported to inhibit immune responsiveness through impairment of IL-2 production; reduction of IL-2 synthesis prevents the clonal expansion of activated T lymphocytes, thus preventing graft rejection (2). We found MicA similarly impaired the production of IL-2 and IL-2 receptor expression of ConA-activated spleen cells in a concentration-dependent fashion.

Moreover, due to its nanomolar level of activity, MicA was compared with the reference standard cyclosporin A and found to be more potent than cyclosporin A in suppressing lymphocyte proliferation induced by most stimuli. Whether MicA and cyclosporin A share a common mode of action remains to be elucidated.

In summary, our studies indicate that MicA possesses antiproliferative and immunosuppressive effects on lymphocytes at concentrations which are not toxic *in vitro*. Further studies are in progress in our laboratory to clarify the exact mechanisms whereby this novel lipopeptide exerts its antiproliferative and immunosuppressive effects.

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