Concanamycin B Inhibits the Expression of Newly-synthesized MHC Class II Molecules on the Cell Surface

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We screened natural chemical compounds which inhibit the expression of newly-synthesized MHC class II molecules on the cell surface. IFN-γ stimulates Colo 205 adenocarcinoma cells to induce the expression of MHC class I, MHC class II, and ICAM-1 molecules on the cell surface. We show here that concanamycin B inhibits the expression of MHC class II molecules on Colo 205 cells, whereas it does not affect the expression of MHC class I and ICAM-1 molecules. Concanamycin B also abrogates the enhancement of the MHC class II expression by IL-4 on mouse lymphocytes. Furthermore, concanamycin B suppresses the antigen presentation by MHC class II molecules.

The 18-membered macrocyclic antibiotic concanamycin B was originally discovered as the inhibitor of the proliferation of splenic lymphocytes by concanavalin A[1]. Recently concanamycin B was elucidated to be an inhibitor of vacuolar H^+-ATPase (V-ATPase) and to inhibit cholesteryl-ester synthesis from oleate[2,3]. The H^+-ATPase in the vacuolar system is responsible for generating the proton gradients for the various stages of endocytic and exocytic pathways[4]. Concanamycin B significantly delays the onset of secretion of the proteins[5].

By screening compounds which inhibit the cell-surface expression of MHC class II molecules, we found that concanamycin B inhibits the expression of functional MHC class II molecules and suppresses the antigen presentation by this molecule. MHC class II molecules play a role of self/non-self discrimination in the immune system and are also involved in the development of autoimmune diseases[6~10]. MHC class II molecules typically assemble in the ER as a stoichiometric complex with a nonpolymorphic invariant chain (Ii), a type II integral membrane protein. This complex is transported out of ER and through the Golgi, undergoing terminal glycosylation during this passage. The MHC class II-Ii complexes then appear to move to lysosome-related compartments. In these compartments the Ii chain is removed from MHC class II molecules by proteases and the peptide antigen processed in the lysosome then binds to the MHC class II molecule. This peptide-bearing dimer transits to the cell surface and stimulates helper T cells. Since acidotropic agents such as chloroquine (CQ) or ammonium chloride inhibit antigen presentation by MHC class II molecules, acidification in organelles is critical for antigen presentation by these molecules[11,12]. We show here that concanamycin B is a more potent and specific inhibitor of the antigen presentation by MHC class II molecules.

Materials and Methods

Preparation of Concanamycin B

Concanamycin B was extracted from the mycelium of Streptomyces sp. AJ9467 with methanol. It was further

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extracted with ethyl acetate at pH 7.5 and purified by silica gel with ethyl acetate and by HPLC on a YMC-Pack D-ODS-5 column with an acetonitrile/water concentration gradient from 60~100%.

Transformations of Concanamycin B

(A) Concanamycin B aglycone

A solution of concanamycin B (4 mg) in aqueous 50% acetic acid (0.2 ml) was stirred at 40°C for 15 hours and then concentrated to give a crude syrup which was chromatographed on silica gel with 1:1 benzene-ethyl acetate to afford the aglycon of concanamycin B.

(B) Alkaline degradation product

A solution of concanamycin B (3 mg) in 0.03 N NaOH-MeOH (0.2 ml) was stirred at 25°C for 10 minutes. The solution was then neutralized with CG-50 resin (H^+ type) under ice-cooling, and the mixture was filtered. The filtrate was concentrated to give a crude product which was chromatographed on silica gel with 2:1 benzene-ethyl acetate to afford alkaline-degradation derivatives (1.1 mg, 46%).

(C) Non-macrocyclic product

To an ice-cooling solution of concanamycin B (4 mg) in dry MeOH (0.2 ml) was added *dl*-10-camphorsulfonic acid (CSA) (0.2 mg). After stirring at 25°C for 30 minutes, the mixture was neutralized with amberlite IRA-400 resin and filtered. The filtrate was concentrated to a crude product which was chromatographed on silica gel with 1:4 benzene-ethyl acetate to afford *O*-methylconcanamycin B (2 mg) in dry CH_2Cl_2 (0.1 ml) was added a solution of 1 M diisobutylaluminum hydride (DIBAL) in CH_2Cl_2 (0.031 ml). After being stirred at 0°C for 30 minutes, to the mixture was added saturated aqueous NH_4Cl. The mixture was extracted with ether and the extracts were concentrated. The residue was purified by PTLC with 4:1 chloroform-methanol to afford the derivative lacking the macrocyclic system.

Assay of V-ATPase

The membrane fraction from Colo 205 cells was prepared as follows: cells were suspended in 20 mM MOPS-Tris (pH 7.0) buffer containing 0.3 M sucrose, 5 mM EDTA, leupeptin (5 μg/ml) and pepstatin A (5 μg/ml). After homogenization, the suspension was centrifuged at $1,000 \times g$ for 7 minutes and the supernatant was again centrifuged at $200,000 \times g$ for 30 minutes. The pellets were washed once and suspended in the same buffer. ATP-dependent H^+-transport in the absence or presence of concanamycin B was assayed by measuring the fluorescence quenching of acridine orange in 20 mM MOPS-Tris (pH 7.0) buffer containing 0.2 M sucrose, 0.1 M KCl, 2 mM $MgCl_2$, 1 mM valinomycin and 5 mM acridineorange (2 ml). 5 nM concanamycin B was added after and before the addition of Mg-ATP. The activity was sensitive to 1 mM *N*-ethylmaleimide and 50 nM bafilomycin A1 but insensitive to 5 μg/ml of oligomycin, 5 mM azide, or 1 mM vanadate. Synaptic vesicles were prepared from rat brain by previously described methods[14,15]. ATP-dependent H^+-transport in the absence or presence of concanamycin B was assayed as above.

Cells and Culture Conditions

The lymphocytes from spleen or lymph node were centrifuged through lympholyte-M (Cedarlane Co.) and washed three times in RPMI-1640 medium, and used for the experiments. A native goat immunoglobulin (NGG) specific T cell clone, 52-2D, was established from BALB/c mice immunized with NGG. Colo 205 adenocarcinoma cells, WEHI-3 cells, and A20-2JB lymphoma cells were cultured in RPMI-1640 supplemented with 10% FCS, 5×10^{-5} M 2-mercaptoethanol, 100 μg/ml glutamine, 100 u/ml penicillin, and 100 u/ml streptomycin.

Immunofluorescent Staining

Cells were stained for phenotypic analysis essentially as described before[13] and analyzed using FACScan (Beckton Dickinson). We used the following antibodies and 2nd-step reagents: anti-HLA-DR mAb B8.12.2, anti-HLA-ABC mAb B9.12.1, anti-ICAM-1 mAb 84H10, anti-Ia^d mAb MK-D6, anti-Ia^k mAb 11~5.2, goat anti-mouse fluorescein-conjugated antibody, and streptavidin-phycoerythrin. Dead cells were stained with propidiumiodide. The results represent the analysis of 5×10^3 cells.

Quantitative Analysis of mRNA

Total cellular RNA was isolated from WEHI-3 cells and cDNA was specifically synthesized with total RNA according to the following. The 20 μl reaction mixture contained an enzyme buffer (50 mM Tris-HCl, 40 mM KCl, 1 mM DTT, 6 mM $MgCl_2$, 0.1 mg/ml BSA, pH 8.3), 20 units of RNasin (Takara Co.), 10 pmol of 3′ PCR primer, 10 mM (each) deoxynucleotide triphosphate, and 200 units of reverse transcriptase. This mixture was incubated for 30 minutes at 37°C and was then diluted with 80 μl of PCR buffer (5 mM KCl/10 mM Tris-HCl/1.5 mM $MgCl_2$/0.01% gelatin, pH 8.3), followed by the addition of 50 pmol of the 3′ primer , 50 pmol of the 5′ primer, and 1 unit of Taq polymerase. The reaction was initiated by denaturing the RNA·cDNA hybrid by heating at 95°C for 30 seconds, annealing the primers at 55°C for 30 seconds, and then extending the primers at 72°C per minutes. Heat denaturation began the cycle over again; the cycle was repeated 25 times by using a programmable heatblock designed and manufactured by Cetus (Emeryville, CA). Oligonucleotides used for this experiment are given below a chain (IA^d): 5′ primer, GTATAACTGTATATCAGTCT, 3′ primer, GGAATCTCAGGTTCCCAGTG, β chain (IA^d): 5′ primer, GGAAACTCCGAAAGGCATTTCGTG, 3′ primer, GAGGAAGATCACCCCAAGCACGCAGCC, Ii chain: 5′ primer, ATGGATGACCAACGCGACCTCATCTCTAAC, 3′ primer, TGCAGAGCTGGCCTCTGTCTTCACAGGGTGA, β actin: 5′ primer, CCATTGAACATGGCATTGTTACCAACTGGG, 3′ primer, CTTCATGGTGCTAGGAGCCAGAGCAGTAA.

T Cell Stimulation with anti-CD3 mAb

The 52-2D T cell clone (1×10^4) was stimulated for 12 hours with anti-CD3 mAb (2C11) cross-linked to Fc receptor on A20-2J cells (1×10^5), which were pretreated with 50 nM concanamycin B for 4 hours. IL-2 activity in the culture supernatant was measured by mouse IL-2 ELISA (Collaborative Research, Boston, MA).

Antigen Presentation Assay

A20-2J cells (1×10^5) were incubated with the indicated concentrations of normal goat immunoglobulin antigen (NGG) at 37°C in a 5% CO_2 incubator for 4 hours in the absence or presence of 50 nM concanamycin B. The cells were then washed three times with medium, following which they were cocultured with an antigen-specific T cell clone, 52-2D (1×10^4), for 12 hours. Antigen presentation was analyzed by measuring the IL-2 in supernatant by IL-2 ELISA.

Results

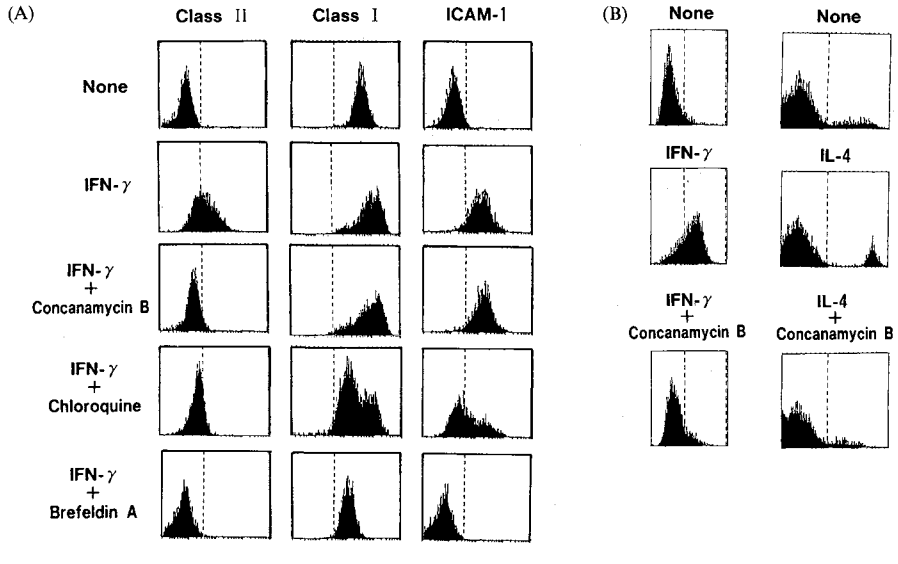
Concanamycin B Inhibits the Expression of Newly Synthesized MHC Class II Molecules

The expression of MHC class II and ICAM-1 molecules on Colo 205 adenocarcinoma cell line was induced by treatment with IFN-γ. The expression of MHC class I molecules on Colo 205 cells was also enhanced by such treatment (Fig. 1-A). By utilizing this system, we screened the natural chemical compounds which specifically inhibited the MHC class II expression. As shown in Fig. 1-A, concanamycin B (50 nM) selectively suppressed the induction of MHC class II molecules, whereas it did not affect the expression of MHC class I and ICAM-1 molecules (Fig. 1-A). We also examined the effect of concanamycin B treatment on the MHC class II expression on mouse myelomonocyte WEHI-3 and mouse splenic cells. As shown in Fig. 1-B, MHC class II molecules were induced on WEHI-3 cells by IFN-γ. Though MHC class II molecules are constitutively expressed on splenic cells, IL-4 treatment enhances the expression. For those cells, concanamycin B again effectively abrogated the induction of MHC class II molecules (Fig. 1-B).

Since both CQ and brefeldin A (BFA) have been demonstrated to suppress cell-surface expression of MHC class II molecules[16,17], therefore, we investigated the effect of CQ and BFA on the induction of MHC class I, MHC class II and ICAM-1 molecules on Colo 205 cells. BFA, which blocks the egress of proteins from ER, completely suppressed the expression of these cell-surface glycoproteins (Fig. 1-A). Since 50 μM CQ partially suppressed the expression of MHC class II molecules without influencing MHC class I and ICAM-1 expression, we increased the concentration of CQ to 200 μM to completely inhibit the expression of MHC class II molecules. However, under this condition it also abrogated the expression of MHC class I and ICAM-1 molecules (Fig. 1-A). These results suggest that concanamycin B is more potent and allows a more specific inhibition of MHC class II expression than CQ or BFA.

Fig. 1. Concanamycin B selectively suppresses the expression of MHC class II molecules.

(A) Colo 205 cells were cultured with hIFN-γ (500 units/ml) in the absence or presence of concanamycin B (50 nM), chloroquine (250 μM) or brefeldin A (1 μg/ml) for 18 hours. (B) WEHI-3 cells and lymph node cells from C3H mice were cultured with mIFN-γ (400 units/ml) or mIL-4 (200 units/ml) in the absence or presence of concanamycin B (50 nM) for 18 hours, respectively. Afterward, MHC class II expression was analyzed.

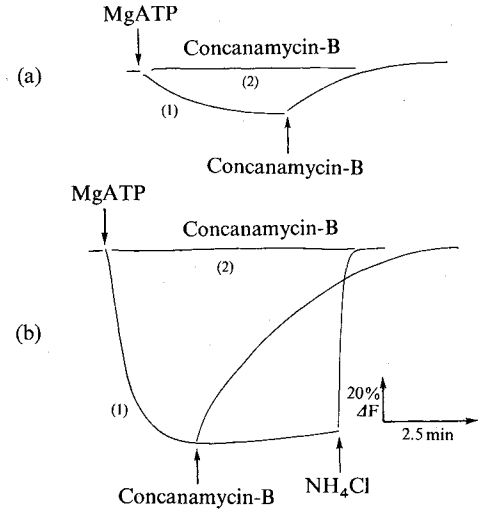


The V-ATPase Inhibition Correlates with the Inhibition of MHC Class II Expression

In order to examine the correlation between V-ATPase activity and MHC class II expression, we generated derivatives of concanamycin B and examined their inhibitory activities. As shown in Fig. 2, concanamycin B inhibits V-ATPase from Colo 205 cells and from synaptic vesicles. We used the V-ATPase from synaptic vesicles for V-ATPase inhibition assay and Colo 205 cells for the assay of MHC class II inhibition. Concanamycin B is an 18-membered macrolide with a long side chain which incorporates a six membered hemiketal ring with a 3-carbamate derivative of 2-deoxy-β-D-rhamnose moiety (Fig. 3-a). Removal of the sugar moiety increased the inhibitory effect on both V-ATPase and MHC class II expression, suggesting the β-D-rhamnose structure to be dispensable for both biological activities (Fig. 3, Table 1). Although alkaline degradation decreased both types of activity, this transformation is not critical. However, hydride reduction of the 18-membered lactone completely destroyed its inhibitory activities for both V-ATPase activity and MHC class II expression (Fig. 3, Table 1). Therefore, the 18-membered macrolide structure in concanamycin B is critical for both biological activities. The inhibition of MHC class II expression should be mediated through V-ATPase inhibition by concanamycin B. We also examined the effect of bafilomycin A1 (Fig. 3-e), which is another V-ATPase inhibitor[18], on the expression of MHC class II molecules. Bafilomycin A1 inhibits the MHC class II expression as well as concanamycin B (Table 1). The structural comparison of the 18-membered macrolide in concanamycin B with the

Fig. 2. Concanamycin B inhibits V-ATPase from Colo 205 cells and synaptic vesicles.

V-ATPase, in Colo 205 cells (a) and synaptic vesicles (b) were inhibited by concanamycin B.



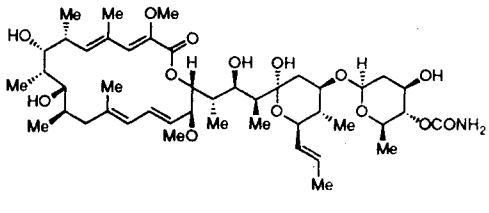
5 nM concanamycin B was added after and before the addition of Mg-ATP for traces 1 and 2, respectively. The activity was sensitive to 1 mM *N*-ethylmaleimide and 50 nM bafilomycin A1 but insensitive to 5 μg/ml of oligomycin, 5 mM azide, or 1 mM vanadate.

Table 1. Inhibition of V-ATPase and expression of MHC class II molecules by the derivatives of concanamycin B.

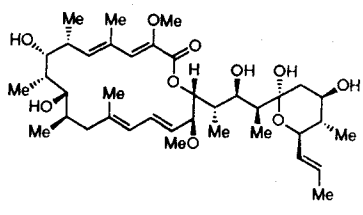
Derivatives	Inhibition IC_{50} (nM)	
	V-ATPase	MHC class II
a	5.0	4.0
b	0.6	0.7
c	9.5	20.0
d	>1000.0	>1000.0
e	4.0	5.0

(a) Concanamycin B, (b) concanamycin B aglycone, (c) alkaline-degradation product, (d) non-macrocyclic product, (e) bafilomycin A1.

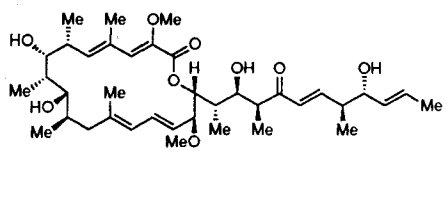
Fig. 3. Chemical structures of bafilomycin A1, concanamycin B and derivatives of concanamycin B.



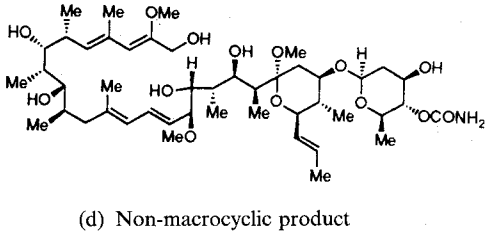
(a) Concanamycin B



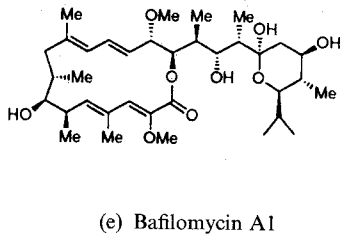
(b) Concanamycin B aglycone



(c) Alkaline degradation product



(d) Non-macrocyclic product



(e) Bafilomycin A1

16-membered macrolide in bafilomycin A1 suggests that two conjugated trans-diene systems might be important for their biological activities.

Concanamycin B does not Inhibit the Transcription of MHC Class II and Ii Genes

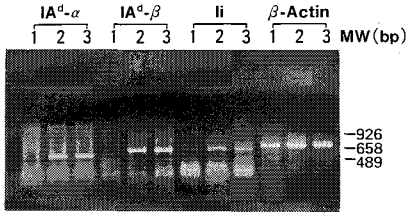
We next examined whether concanamycin B influences transcription of MHC class II α, β, and invariant Ii chain genes in WEHI-3 cells. Total RNA prepared from cells was reverse transcribed and cDNAs from transcripts were amplified under the condition of quantitative PCR. The transcription of MHC class II α, β, and Ii chain genes was induced by IFN-γ. Concanamycin B did not affect their transcription (Fig. 4). Concanamycin B, therefore, blocks MHC class II expression post-transcriptionally.

Concanamycin B Inhibits Antigen Presentation by MHC Class II Molecules

Since it has recently been shown that physiologically processed products derived from exogenous antigens bind to the newly maturing population of MHC class II molecules rather than binding to the preexisting cell surface population[19~21], therefore we investigated the effect of concanamycin B on antigen presentation by MHC class II molecules.

We utilized A20-2J B lymphoma cells as antigen presenting cells and a normal goat immunoglobulin (NGG) specific T cell clone, 52-2D, as responder cells. When NGG antigen is incubated with antigen presenting cells, antigen is internalized into the cells by endocytosis and processed to antigen peptide. This antigen peptide binds to MHC class II molecules in the cells and they are transported to the cell surface. The antigen presented by the MHC class II molecules stimulate helper T cells to proliferate and produce IL-2. TCR associates with CD3 glycoprotein which is necessary for the surface expression of TCR and the signal transduction. Anti-CD3 mAb are strongly mitogenic under appropriate condition[22~24]. At first, we tested the effect of concanamycin B on the production of IL-2 by 52-2D T cells. Anti-CD3 mAb cross-linked to Fc receptor on A20-2J cells stimulates T cells to proliferate and produce IL-2 as antigen presented by MHC class II molecules does. We used cyclosporin A for control experiment[25,26]. Cyclosporin A inhibits the production of IL-2 without the influence on T cell proliferation. However, concanamycin B did not affect the production of IL-2 even if it inhibits the proliferation of T cells (Fig. 5-A). These data eliminate the possibility that concanamycin B has some effect on antigen-induced IL-2 production by T cells. Therefore, we examined the antigen presentation by measuring the IL-2 production after T cells were cultured with antigen presenting cells. As shown in Fig. 5-B, antigen presentation was carried out in an antigen-

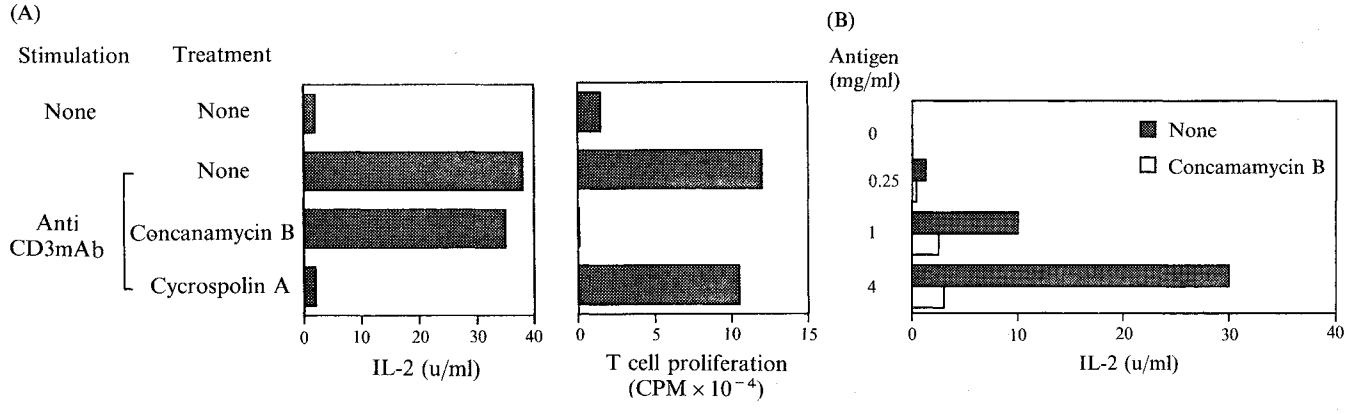
Fig. 4. The effects of concanamycin B on the transcription of MHC class II (α, β) and Ii genes.



Lanes 1, 2, and 3 represent the following treatments: None, mIFN-γ (500 units/ml, 24 hours), and mIFN-γ plus 50 nM concanamycin B (24 hours), respectively. Total cellular RNA was isolated from WEHI-3 cells. cDNA was specifically synthesized with 1 μg of total RNA.

Fig. 5. The effect of concanamycin B on the production of IL-2, proliferation of T cells, and antigen presentation.

(A) Concanamycin B does not inhibit the IL-2 production by 52-2D T cells stimulated with anti-CD3 mAb cross-linked to A20-2J B lymphoma cells even if it inhibits the proliferation of 52-2D T cells. (B) Concanamycin B inhibits the antigen presentation by A20-2J B lymphoma cells.



dependent fashion, whereas antigen presentation by A20-2J cells was abrogated in the presence of concanamycin B. These results suggest that concanamycin B inhibits antigen presentation.

Discussion

Based on the chemical structure of active site in concanamycin B for inhibition of MHC class II expression, we found that leucanicidin[27] or hygrolidin[28] have the same inhibitory activities as concanamycin B (data not shown). We also examined the effect of FK506 and cycrospolin A on the inhibition of MHC class II expression. Those immunosuppressors didn't affect the MHC class II expression. Acidtropic agents such as chloroquine, ammonium chloride, and ionophore monensin require higher concentration to inhibit MHC class II expression and their specificity is lower than concanamycin B. Concanamycin B is a potent inhibitor of MHC class II expression.

How does concanamycin B inhibit the expression of MHC class II molecules? The biosynthesis of MHC class II molecules starts with the assembly of α and β subunits and the Ii chain. In the course of intracellular transport, the MHC class II molecules associated with Ii chain is removed in the endosome pathway and finally MHC class II α-β heterodimer is expressed on the cell. Since concanamycin B affect the post-transcriptional biosynthesis of MHC class II molecules (Fig. 4), we examined the effect of concanamycin B on the intracellular transport of MHC class II molecules. Dissociation of Ii chain from MHC class II molecule was inhibited by concanamycin B treatment and most of the MHC class II α,β heterodimer associated with Ii chain were accumulated inside the cell[29]. Interestingly concanamycin B treatment increases the expression of Ii and Ii-MHC class II $\alpha\beta$ complex on the cell surface[29]. Therefore, concanamycin B inhibits the transport of MHC class II molecules to the cell surface by neutralization of vacuolar compartments which prevents the dissociation of Ii chain.

Regarding the inhibition of antigen presentation by concanamycin B, we believed that concanamycin B is most likely involved in preventing the formation of mature MHC class II complexes and proper processing of antigens. Since the acidification is important for the phagocytosis and endocytosis of antigen[30], the uptake of antigen into antigen-presenting cells may be thwarted. In addition, concanamycin B may also inhibit the degradation of exogenous antigens in the endosomes and lysosomes by elevating the pH, because an acidic pH 3~4 is necessary for optimal activity of the proteases involved in degradation. As described above, concanamycin B obstructs the dissociation of the Ii chain from the MHC class II molecules. As a result the Ii chain which is still associated with the MHC class II molecules might interfere with proper binding of a peptide. However, even if the antigenic peptides are able to bind to this immature Ii-MHC class II molecule the antigen probably still can not be presented due to the decreased ability of the complex to be transported to the cell surface or T cells can not recognize the antigen presented by the immature Ii-MHC class II complex.

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References

1) KINASHI, H.; K. SOMENO & K. SAKAGUCHI: Isolation and characterization of concanamycin A, B, and C. J. Antibiotics 37: 1333~1343, 1984
2) WOO, J. T.; C. SHINOHARA, K. SAKAI, K. HASUMI & A. ENDO: Isolation, characterization and biological activities of concanamycins as inhibitors of lysosomal acidification. J. Antibiotics 45: 1108~1116, 1992
3) WOO, J. T.; C. SHINOHARA, K. SAKAI, K. HASUMI & A. ENDO: Inhibition of the acidification of endosomes and lysosomes by the antibiotic concanamycin B in macrophage J774. Eur. J. Biochem 207: 383~389, 1992
4) MELLMAN, I.; R. FUCHS & A. HELENIUS: Acidification of the endocytic and exocytic pathway. Ann. Rev. Biochem. 55: 663~700, 1986
5) YILLA, M.; A. TAN, K. ITO, K. MIWA & H. L. PLOEGH: Involvement of the vacuolar H^+-ATPase in the secretory pathway of HepG2 cells. J. Biol. Chem. 268: 19092~19100, 1993
6) BENACERRAF, B. & H. O. MCDEVITT: Histocompatibility-linked immune response genes. Science 175: 273~279, 1972
7) SCHWARTZ, R. H.: T-lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. Annu. Rev. Immunol. 3: 237~261, 1985
8) TODD, J. A.; H. ACHA-ORBEA, J. I. BELL, N. CHAO, Z. FRONEK, C. O. JACOB, M. MCDERMOTT, A. A. SINHA, L. TIMMERMAN, L. STEINMAN & H. O. MACDEVITT: A molecular basis for MHC class II associated autoimmunity. Science 240: 1003~1009, 1988
9) WATANABE, Y.; K. TOKUNAGA, K. MATSUKI, F. TAKEUCHI, K. MATSUTA, H. MAEDA, K. OMOTO & T. JUJI: Putative amino acid sequence of HLA-DRβ chain contributing to Rheumatoid Arthritis susceptibility. J. Exp. Med. 169: 2263~2268, 1989
10) SVEJGAARD, A.; P. PLATZ & L. P. RYDER: HLA and disease 1982-a survey. Immunol. Rev. 70: 193~218, 1983
11) GUIDOS, C.; M. WONG & K. C. LEE: A comparison of the stimulatory activities of lymphoid dendritic cells and macrophages in T proliferative responses to various antigens. J. Immunol. 133: 1179~1184, 1984
12) GREY, H. & R. CHESNUT: Antigen processing and presentation to T cells. Immunol. Today 6: 101~106, 1985
13) BONNEVILLE, M.; K. ITO, E. G. KRECKO, S. ITOHARA, D.

KAPPES, I. ISHIDA, O. KANAGAWA, C. A. JANEWAY, Jr., D. B. MURPHY & S. TONEGAWA: Recognition of a self MHC TL region product by $\gamma\delta$ T cell receptors. Proc. Natl. Acad. Sci. USA 86: 5928~5932, 1990

14) HUTTNER, W. B.; W. SCHIEBLER, P. GREENGARD & P. DE CAMILLI: Synapsin 1 (protein 1), a nerve terminal-specific phosphoprotein III. Its association with synaptic vesicles studied in a highly purified synaptic vesicle preparation. J. Cell. Biol. 96: 1374~1388, 1983

15) KISH, P. E.; C. FISCHER-BORENKERK & T. UEDA: Active transport of r-aminobutyric acid and glycine into synaptic vesicles. Proc. Natl. Acad. Sci. USA 86: 3877~3881, 1989

16) NOWELL, J. & V. QUARANTA: Chloroquine affects biosynthesis of Ia molecules by inhibiting dissociation of invariant (r) chains from α-β dime in B cells. J. Exp. Med. 162: 1371~1376, 1985

17) ADORINI, L.; S. J. ULLRICH, E. APPELLA & S. FUCHS: Inhibition by brefeldin A on presentation of exogenous protein antigens to MHC class II-restricted T cells. Nature 346: 63~66, 1990

18) BOWMAN, E. J.; A. SIEBERS & K. ALTERDORF: Bafilomycins: A class of inhibition of membrane ATPases from microorganisms, animal cells, and plant cells. Proc. Natl. Acad. Sci. USA 85: 7972~7976, 1988

19) DAVIDSON, H. W.; P. A. REID, A. LANZAVECCHIA & C. WATTS: Processed antigen binds to newly synthesized MHC class II molecules in antigen-specific B lymphocytes. Cell 67: 105~116, 1991

20) PETERS, P. J.; J. J. NEEFJES, V. OORSCHOT, H. L. PLOEGH & H. J. GEUZE: Segregation of MHC class II molecules from MHC class I molecules in the Golgi complex for transport to lysosomal compartments. Nature 349: 669~676, 1991

21) NEEFJES, J. J.; V. STOLLORZ, P. J. PETER, H. J. GEUZE & H. L. PLOEGH: The biosynthetic pathway of MHC class II but not class I molecules intersects the endocytic route. Cell 61: 171~183, 1990

22) SPRENT, J. & S. R. WEBB: Function and specificity of T cell subsets in the mouse. Adv. Immunol. 41: 39~133, 1987

23) CLEVERS, H.; B. ALARCON, T. WILEMAN & C. TERHOLST: The T cell receptor/CD3 complex : A dynamic protein ensemble. Annu. Rev. Immunol. 6: 629~662, 1988

24) LEO, O.; M. FOO, D. SACHS, L. E. SAMELSON & J. A. BLUESTONE: Identification of a monoclonal antibody specific for a murine T3 polypeptide. Proc. Natl. Acad. Sci. USA 84: 1374~1378, 1987

25) SHEVACH, E. M.: The effect of cyclosporin A on the immune system. Annu. Rev. Immunol. 3: 397~423, 1985

26) KUMAGAI, N.; S. H. BENEDICT, G. B. MILLS & E. W. GELFAND: Cyclosporin A inhibits initiation but not progression of human T cell proliferation triggered by phorbol esters and calcium ionophores. J. Immunol. 141: 3747~3752, 1988

27) ISOGAI, A.; S. SAKUDA, S. MATSUMOTO, M. OGURA, K. FURIHATA, H. SETO & A. SUZUKI: The structure of leucanicidin a novel insecticidal macrolide produced by *Streptomyces halstedii*. Agric. Biol. Chem. 48: 1379~1381, 1984

28) GOET, M. A.; P. A. MCCORMICK, R. L. MONAGHAN, D. A. OSTLIND, O. D. HENSENS, J. M., LIESCH & G. ALBERS-SCHONBERG: L-155,175: A new antiparasitic macrolide fermentation, isolation and structure. J. Antibiotics 38: 161~168, 1985

29) BE'NAROCH, P.; M. YILLA, G. RAPOSO, K. ITO, K. MIWA, H. J. GEUZE & H. L. PLOEGH: How MHC class II molecules reach the endocytic pathway. EMBO J. 14: 37~49, 1995

30) LUKACS, G. L.; O. D. ROTSTEIN & S. GRINSTEIN: Phagosomal acidification is mediated by a vacuolar-type H^+-ATPase in murine macrophages. J. Biol. Chem. 265: 21099~21107, 1990