Inhibitory Mechanism of Mizoribine on the Antibody Production of Mouse B Cells Stimulated with Lipopolysaccharide

Hiroyuki Kamada1, Hiromichi Itoh1, Hiroshi Shibata1, Takehiro Koshio1, Akira Hayashi2 and Keiji Nakagami1

1Laboratory for Immunology and 2Laboratory for Chemistry, Institute for Life Science Research, Asahi Chemical Industry Co., Ltd., 2-1 Samejima, Fuji, Shizuoka 416, Japan

Received January 28, 1997 Accepted May 31, 1997

ABSTRACT—It has been reported that the immunosuppressant mizoribine (MZR) inhibits T cell proliferation by depleting intracellular guanine nucleotides via competitive inhibition of inosine 5'-monophosphate (IMP) dehydrogenase in the purine metabolism pathway. This study was performed to determine if the mechanism by which MZR suppresses the proliferation of mouse B cells and antibody production by these cells is dependent on the depletion of intracellular guanine nucleotides. Stimulation of purified splenic B cells of mice with lipopolysaccharide (LPS), a mitogen to B cells, increased both proliferation and antibody production. MZR suppressed both of these functions in a dose-dependent fashion. MZR also caused a decrease in the amount of intracellular guanosine 5'-triphosphate (GTP). When the cultures were grown on plates containing guanosine plus 8-aminoguanosine, the amount of intracellular GTP, which had been reduced by MZR, was restored. Furthermore, the repletion of GTP pools restored both proliferation and antibody production. MZR suppressed both of these functions in a dose-dependent fashion. MZR also caused a decrease in the amount of intracellular guanosine 5'-triphosphate (GTP). These results suggest that MZR suppresses antibody production and proliferation of B cells by acting directly on B cells. Furthermore, it is suggested that the inhibitory effect of MZR on antibody production, as well as on T cell proliferation, is dependent on the decrease in intracellular guanine nucleotide pools of mouse B cells.

Keywords: Mizoribine, GTP level, B cell, Antibody production

Mizoribine (MZR), an immunosuppressive agent, is an imidazole nucleoside isolated from the culture filtrate of Eupenicillium brefeldianum (1); it suppresses both humoral and cellular immune responses (2, 3). MZR has been clinically used mainly for kidney transplantation (4–6) and recently for treatment of lupus nephritis (7) and rheumatoid arthritis (8).

MZR suppresses antibody production to both T cell-dependent antigens and T cell-independent antigens in a dose-dependent fashion both in vivo and in vitro (2, 3, 9, 10). MZR suppresses the proliferation of lymphocytes induced by lipopolysaccharide (LPS), a mitogen to B cells, as well as that induced by concanavalin A (Con A), a mitogen to T cells (2, 9). It is known that MZR inhibits not only the growth of T cells but also that of B cells.

Studies of MZR with mouse lymphoma L5178Y cells have shown that MZR is converted to its monophosphate form by adenosine kinase (11) and inhibits inosine 5'-monophosphate (IMP) dehydrogenase in the purine metabolism pathway, resulting in depletion of intracellular guanine nucleotides (12). Furthermore, the co-existence of guanosine 5'-monophosphate (GMP) restores MZR-induced suppression of L5178Y cell proliferation (13, 14). Recently, Mitchell et al. (15, 16) investigated the effects of MZR on human peripheral blood T cells stimulated with alloantigen or pharmacologic mitogens. They demonstrated that MZR-induced suppression of the T cell proliferation was associated with a decrease in the amount of intracellular guanosine 5'-triphosphate (GTP). These results suggest that the depletion of intracellular guanine nucleotide plays a role in MZR-induced suppression of cell proliferation. In this paper, we examined the suppressive effect of MZR on antibody production and proliferation of purified mouse splenic B cells.

MATERIALS AND METHODS

Animals

Male ICR mice were obtained from Japan SLC, Inc., Hamamatsu and were used at 6–7 weeks of age.
Chemicals
The sources of materials used in this study were as follows: anti-Thy1.2 monoclonal antibody (250 μg/ml) from Becton Dickinson, San Jose, CA, USA; LPS (from Salmonella typhimurium), guanosine (GR), 8-amino-guanosine (8-A-GR) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma Chemical, St. Louis, MO, USA; Hanks' balanced salt solution (HBSS) and RPMI 1640 medium from Nissui Pharmaceutical, Tokyo; sheep red blood cells (SRBC) from Nippon Bio-Test Laboratories, Inc., Tokyo; MZR from Asahi Chemical Industry, Osaka. Guinea pig serum, adsorbed with mouse splenocytes in advance, was used as the source of complements.

Purification and culture of B cells
Single cell suspensions of splenocytes were passed through a stainless steel sieve in HBSS. After treatment with Tris-buffered ammonium chloride to remove erythrocytes, the splenocytes were washed and suspended at a density of 1 × 10⁷/ml in RPMI 1640 medium supplemented with 10 mM HEPES. Six microliters of anti-Thy1.2 monoclonal antibody solution was added to 1 ml of the cell suspension, and the mixture was incubated at 4°C for 30 min with occasional shaking. After washing, the splenocytes were resuspended at a density of 1 × 10⁷/ml in HBSS containing 5% guinea pig serum and incubated at 37°C for 60 min with occasional shaking. The purified B cells at a density of 2 × 10⁶/ml were cultured at 37°C in a humidified atmosphere of 5% CO2 for 3-4 days in 12-well microplates (Iwaki Glass, Tokyo) with or without LPS. MZR and GR plus 8-A-GR were added simultaneously with LPS. The culture medium used here was RPMI 1640 supplemented with 10% fetal calf serum (FCS; Hyclone Laboratories, Inc., Logan, UT, USA), 2 mM L-glutamine, 5 × 10⁻² M 2-mercaptopethanol, 100 U/ml penicillin and 100 μg/ml streptomycin.

MTT assay
The cultured and resuspended B cells were transferred to 96-well microplates (Nunc, Roskilde, Denmark) in a volume of 100 μl. The MTT assay was performed as described by Mosmann (17). Briefly, 10 μl of 5 mg/ml MTT dissolved in phosphate-buffered saline (PBS) was added to each well, and the microplates were further incubated at 37°C for 6 hr. Acidified isopropanol (0.04 N HCl in isopropanol) was added to the cultures and mixed thoroughly to dissolve the dark blue crystals of formazan. Formazan quantification was performed with a microplate photometer MTP-22 (Corona Electric, Ibaraki) with a 577 nm test wavelength and a 630 nm reference wavelength.

Anti-SRBC plaque forming cell (PFC) assay
At the end of the incubation, anti-SRBC PFC in the cultured B cells was measured according to the method of Cunningham and Szenberg (18). Briefly, 0.05 ml of SRBC (30% suspension) and 0.05 ml of guinea pig serum were added to 0.4 ml of the cultured B cell suspension and the mixture was filled in a Cunningham's chamber (Takahashi Giken Glass Work's, Tokyo). After incubation at 37°C for 60 min, the plaques were counted.

IgM antibody in culture supernatant
Culture supernatants were obtained at the end of the incubation. The amount of IgM antibody in the culture supernatant was measured by the enzyme-linked immunosorbent assay (ELISA) technique. Briefly, 96-well immunoplates (Nunc) were coated with 50 μl of antimouse IgM monoclonal antibody (purified from nude mouse ascites), diluted 1 : 1000, and incubated overnight at 4°C. The plates were washed three times with PBS containing 0.05% Tween 20 (PBS-Tween) and blocked with PBS containing 1% bovine serum albumin (PBS-1%BSA). After incubation for 1 hr at room temperature, the plates were washed three times with PBS-Tween. Fifty-microliter aliquots of the culture supernatant, diluted 1 : 128 with PBS-1%BSA, were added to each well. After incubation for 1 hr at room temperature, the plates were washed four times with PBS-Tween. Fifty-microliter of 1 : 2000 dilution of peroxidase-conjugated goat anti-mouse IgM(μ) (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA) were added. After an additional incubation for 1 hr at room temperature, the plates were washed four times with PBS-Tween. Fifty-microliter of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Wako Pure Chemical Industries, Osaka) solution were added as the substrate. After incubation for 30 min at 37°C, the reaction was stopped by adding 50 μl of 5% oxalic acid. The reaction product was measured by determining the absorbance at 415-492 nm with a microplate photometer (MTP-22). IgM antibody levels were shown as the values converted per 10⁷ cells (of B cells).

Measurement of intracellular nucleotide pools
At the end of the incubation, cells were collected and washed with PBS. The cells were treated with ice-cold 0.4 N perchloric acid for 5 min at 4°C to extract nucleotides, and then they were centrifuged at 13,000 × g for 10 min. The supernatants were neutralized with 1 N KOH and centrifuged again to remove precipitate including salts. The resulting extracts were stored at -20°C until use. Nucleotide pools were quantified using HPLC with ATP and GTP standards (Sigma Chemical) under the conditions described below:
Systems: Shimadzu LC-6A pump GR system
(Shimadzu, Tokyo)
Column: Shodex AXpak WA-624 (6 mm I.D. × 150)
(Showa Denko, Tokyo)
Diluent A: 15% CH₃CN in 0.1 M NaH₂PO₄
(0–15 min)
B: 30% CH₃CN in 0.5 M NaH₂PO₄
(15–35 min)
Flow rate: 1.0 ml/min
Detector wavelength: UV-260 nm
Column temperature: 50°C
Amounts of the intracellular nucleotides were shown as the values converted per 10⁷ cells.

RESULTS

Proliferation, non-specific antibody production and intracellular GTP pools of B cells stimulated with LPS

Purified B cells used here were obtained by treating splenocytes with anti-Thy1.2 antibody and complement. Viability of recovered cells was more than 98% and total recovery of the viable cells after this treatment was 57–63%. It was thought that most of the T cells had been removed because the proliferative response of the recovered cells stimulated with Con A was reduced to less than 5% as compared to that of non-treated splenocytes (data not shown). The B cells were cultured with LPS at various concentrations for 3 days (Fig. 1). Their prolifera-
tion was enhanced by stimulation with LPS at 1.25–10 μg/ml in a dose-dependent fashion as indicated by both the cell number and MTT assay. Amount of IgM antibody produced by 10⁷ cells in the culture supernatant also increased threefold as much as that of non-stimulated cells. The amount of intracellular GTP was slightly increased by stimulation with LPS at concentrations of 2.5–10 μg/ml.

Suppressive effect of MZR on proliferation, non-specific antibody production and intracellular GTP pools of B cells stimulated with LPS

We examined the effect of MZR on B cells stimulated with LPS at a concentration of 5 μg/ml (Fig. 2). MZR suppressed LPS-induced B cell proliferation at concentrations of more than 1.0 μg/ml and also suppressed antibody production in a dose-dependent fashion at 0.25 – 2.0 μg/ml. Moreover, MZR decreased the amount of intracellular GTP in a dose-dependent fashion. Thus MZR suppressed both B cell proliferation and antibody production, while it decreased the amount of intracellular GTP. However, there was a difference in the concentrations of MZR to suppress these parameters. A concentration of 0.5 μg/ml MZR did not suppress B cell proliferation, but MZR at the same concentration suppressed antibody production and decreased the amount of intracellular GTP to 43% and 39%, respectively, compared to the values of the control. Consequently, MZR suppressed antibody production at concentrations that were too low to suppress B cell proliferation.

Fig. 2. Effect of MZR on proliferation, non-specific antibody production and intracellular GTP pools of B cells stimulated with LPS. The purified B cells were stimulated with 5 μg/ml LPS in the presence of MZR at various concentrations. After incubation for 3 days, each B cell function was examined as described in the legend of Fig. 1. Results are expressed as mean values of duplicate wells.
Fig. 3. Effect of combined treatment of GR and 8-A-GR on decrease in intracellular GTP pools in B cells by MZR. The purified B cells were stimulated with 5 µg/ml LPS in the absence (open columns) or presence (solid columns) of 2 µg/ml MZR for 3 days. Cultures were supplemented with GR plus 100 nM 8-A-GR. Results are expressed as mean values of duplicate wells. Figures above columns show percentages compared to the values of each MZR-untreated culture.

Restoration of the amount of intracellular GTP from MZR-induced depression by addition of GR plus 8-A-GR

The above results show that MZR suppressed proliferation and antibody production of B cells, while it decreased the amount of intracellular GTP in those cells. Then we added GR plus 8-A-GR to culture medium to restore MZR-induced depletion of the amount of intracellular GTP (Fig. 3). A concentration of 2.0 µg/ml MZR decreased the amount of intracellular GTP to 12% as compared to the value in MZR-untreated cells. The addition of GR plus 8-A-GR led to restoration of the amount of intracellular GTP; i.e., the amounts of intracellular GTP decreased by MZR were restored by the addition of 50 µM GR plus 100 µM 8-A-GR and 100 µM GR plus 100 µM 8-A-GR to 46% and 71%, respectively, as compared to the value in each MZR-untreated cells.

Restoration of B cell proliferation and antibody production following MZR-induced suppression by addition of GR plus 8-A-GR

Next, we examined restoration of B cell functions following suppression by MZR. B cell proliferation which was suppressed to 33% and 31% of the level of untreated cells by 2.0 µg/ml MZR, as measured by cell number and MTT assay, respectively, was restored to 63–78% by the addition of GR plus 8-A-GR (Fig. 4). We also examined whether the antibody production was restored by the addition of GR plus 8-A-GR. The amounts of IgM antibody in the culture supernatant on day 4 were decreased by MZR at concentrations of 1.0 and 2.0 µg/ml to 50% and 13% of the value of MZR-untreated cells, respectively. But the addition of 50 µM or 100 µM GR plus 8-A-GR restored the levels to more than 80% and more than 90%, respectively (Fig. 5). Stimulation of B cells with LPS generated anti-SRBC PFC, and this response was also suppressed by MZR in a dose-dependent fashion. This immune response was similarly restored by the addition of GR plus 8-A-GR.

Fig. 4. Effect of combined treatment of GR and 8-A-GR on suppression of proliferation of B cells by MZR. The purified B cells were stimulated with 5 µg/ml LPS in the absence (open columns) or presence (solid columns) of 2 µg/ml MZR for 3 days. Cultures were supplemented with GR plus 100 µM 8-A-GR. Results are expressed as mean values of duplicate wells. Figures above columns show percentages compared to the values of each MZR-untreated culture. A: Cell number, B: MTT assay.
DISCUSSION

In order to clarify the inhibitory mechanism of MZR on the antibody production, we have studied the relationship between MZR-induced suppression of antibody production and the amount of intracellular guanine nucleotide in mouse B cells.

Stimulation of mouse B cells with LPS led to cell proliferation accompanied by non-specific antibody production (Fig. 1). This proliferative response was suppressed by MZR in a dose-dependent fashion, and MZR decreased the amount of IgM antibody in the culture supernatant and the amount of intracellular GTP (Fig. 2). Therefore, it was thought that the suppression of antibody production in mouse B cells was a result of a decrease in the amount of GTP caused by MZR. We then examined whether MZR-induced suppression of B cell functions would be restored by addition of GR plus 8-A-GR. It has been previously shown that the combination of GR and 8-A-GR results in a sustained increase in the amount of intracellular GTP, whereas GR alone results in more transient increases in cultured T and B lymphoblasts (19). This effect is mediated by the slow conversion of GR to guanine in the presence of 8-A-GR, a weak competitive inhibitor of the degradatory enzyme purine nucleoside phosphorylase (20). The amount of intracellular GTP decreased by MZR increased considerably following the addition of GR plus 8-A-GR (Fig. 3). Therefore, MZR-induced suppression of the proliferative response was recovered with GTP repletion (Fig. 4). It is reported that the combination of GR and 8-A-GR augmented the stimulated human T cell proliferation (15). However, the addition of GR and 8-A-GR suppressed the stimulated mouse B cell proliferation in a dose-dependent fashion (Fig. 4). The definite reason for the suppression of B cell proliferation by GTP repletion is not clear, but mouse B cells may be more sensitive to GR and the cell proliferation may be suppressed by exposure to a high concentration of GR. MZR-induced suppression of the antibody production, i.e., anti-SRBC PFC response and the IgM production, of the B cells was similarly restored by the addition of GR plus 8-A-GR, like the proliferative response was (Fig. 5). Okubo et al. (21) reported that MZR suppressed the antibody production of mouse splenocytes, but that the addition of GMP could not reverse this suppression. However, they did not investigate whether the amount of intracellular guanine nucleotide in the cells was restored by addition of GMP. We found that the addition of GMP restored antibody production of mouse splenocytes from MZR-induced suppression (data not shown) and that MZR-induced suppression of antibody production of mouse splenocytes was dependent on the depletion of intracellular guanine nucleotide pools. Therefore, it is suggested that the inhibitory mechanism of action of MZR on the antibody production of mouse B cells is dependent on the depletion of intracellular guanine nucleotide pools.

Two previous reports describe the effect of MZR on human B cells. Terai et al. (22) demonstrated the inhibitory effect of MZR on the proliferation of Epstein-Barr virus transformed human B cell lines. Hirohata and Yanagida (23) also reported that MZR suppressed IgM production of purified human B cells stimulated with Staphylococcus aureus Cowan I (SA) plus interleukin (IL)-2. These two reports showed that MZR directly affects human B cells and inhibits the functions, the
proliferation and antibody production, of those cells as well as mouse B cells. With regard to the inhibitory mechanism of MZR on human B cells, Terai et al. suggested that a decreased supply of guanine nucleotides is the main mechanism underlying the growth inhibitory effects of MZR as addition of guanine rescued human B cells from growth inhibition by MZR, being consistent with our present report using mouse B cells. On the other hand, Hirohata and Yanagida suggested that MZR suppresses human B cell functions by a mechanism distinct from GTP depletion because the antibody production of human B cells was not reversed by the addition of GMP or GR plus 8-A-GR. In those reports and our present report, there is a difference in the amount of intracellular GTP in B cells. The amount of GTP in transformed human B cell line and that in LPS-stimulated mouse B cells was about 5500 pmol/10^7 cells (22) and about 1800 pmol/10^7 cells, respectively. However, the amount of GTP in SA + IL-2-stimulated human B cells was 80–180 pmol/10^7 cells (23) and, therefore, was much lower than that in transformed human B cell line and LPS-stimulated mouse B cells. By the way, the amount of GTP in human T cells, stimulated with a combination of phorbol myristate acetate, ionomycin plus anti-CD28 antibody, was about 4800 pmol/10^7 cells (15). It is demonstrated that MZR-induced suppression of this human T cell proliferation is dependent on the depletion of intracellular GTP pools (15). It is suggested that MZR-induced suppression of the proliferation and the antibody production of B cells containing a high amount of GTP is dependent on a mechanism of GTP depletion but that of B cells containing a low amount of GTP is dependent on a mechanism distinct from GTP depletion. There is another possibility that the metabolism of guanine nucleotide in human B cells might be differently regulated from that in mouse B cells. Further clarification of this mechanism is needed.

In summary, we showed that MZR suppresses the proliferation and the antibody production of mouse B cells by directly affecting B cells. It is suggested that the inhibitory effect of MZR on the antibody production, as well as on the proliferation of T cells, is dependent on the depletion of intracellular guanine nucleotide pools of mouse B cells.

References

1. Mizuno K, Tsujino M, Takada M, Hayashi M, Atsumi K, Asano K and Matsuda T: Studies on bredinin: Isolation, characterization and biological properties. J Antibiot (Tokyo) 27, 775–782 (1974)
2. Kamata K, Okubo M, Ishigamori E, Masaki Y, Uchida H, Watanabe K and Kashiwagi N: Immunosuppressive effect of bredinin on cell-mediated and humoral immune reactions in experimental animals. Transplantation 35, 144–149 (1983)
3. Okubo M, Chen X-M, Kamata K, Masaki Y and Uchiyama T: Suppressive effect of mizoribine on humoral antibody production in DBA/2 mice. Transplantation 41, 495–498 (1986)
4. Tajima A, Hata M, Ohia N, Ohtawara Y, Suzuki K and Aso Y: Bredinin treatment in clinical kidney allografting. Transplantation 38, 116–118 (1984)
5. Aso K, Uchida H, Sato K, Yokota K, Osakabe T, Nakayama Y, Ohkubo M, Kumano K, Endo T, Koshih K, Watanabe K and Kashiwagi N: Immunosuppression with low-dose cyclosporine combined with bredinin and prednisolone. Transplant Proc 19, 1955–1958 (1987)
6. Osakabe T, Uchida H, Masaki Y, Yokota K, Sato K, Nakayama Y, Ohkubo M, Kumano K, Endo T, Watanabe K and Aso K: Studies on immunosuppression with low-dose cyclosporine combined with mizoribine in experimental and clinical cadaveric renal allotransplantation. Transplant Proc 21, 1598–1600 (1989)
7. Kondo H, Okada J and Kashiwazaki S: Clinical trial of long-term administration of mizoribine in the treatment of lupus nephritis. Clin Immunol 21, 643–650 (1989) (Abstr in English)
8. Shiokawa Y, Henma M, Shichikawa K, Miyamoto T, Hirose J, Nobunaga T, Mizushima Y, Sugawara S, Warabi H, Kondo H and Ogawa N: Clinical effectiveness of mizoribine on rheumatoid arthritis: A double-blind comparative study using lobezarit disodium as a standard drug. Jpn J Inflamm 11, 375–396 (1991) (Abstr in English)
9. Yata K, Tominaga K, Sone S and Tsubura E: Immunosuppressive effect of 4-carbamoyl-1-β-D-ribofuranosyl imidazol-5-olate (bredinin) in mice. Tokushima J Exp Med 29, 63–69 (1982)
10. Kamada H, Inamura M, Itoh H, Shibata H and Nakagami K: Distinction of optimum time of suppression between mizoribine and cyclosporin A, and synergetic effect on antibody production in mice. Jpn J Pharmacol 58, Supp J, 122P (1992)
11. Koyama H and Tsuji M: Genetic and biochemical studies on the activation and cytotoxic mechanism of bredinin, a potent inhibitor of purine biosynthesis in mammalian cells. Biochem Pharmacol 32, 3547–3553 (1983)
12. Kosumi T, Tsuda M, Katsuunma T and Yamamura M: Dual inhibitory effect of bredinin. Cell Biochem Funct 7, 201–204 (1988)
13. Sakaguchi K, Tsujino M, Yoshizawa M, Mizuno K and Hayano K: Action of bredinin on mammalian cells. Cancer Res 35, 1643–1648 (1975)
14. Sakaguchi K, Tsujino M, Hayashi M, Kawai K, Mizuno K and Hayano K: Mode of action of bredinin with guanlyc acid on L5178Y mouse leukemia cells. J Antibiot (Tokyo) 29, 1320–1327 (1976)
15. Turk a LA, Dayton JS, Sinclair G, Thompson CB and Mitchell BS: Guanine ribonucleotide depletion inhibits T cell activation: Mechanism of action of the immunosuppressive drug mizoribine. J Clin Invest 87, 940–948 (1991)
16. Dayton JS, Turk a LA, Thompson CB and Mitchell BS: Comparison of the effects of mizoribine with those of azathioprine, 6-mercaptopurine, and mycophenolic acid on T lymphocyte proliferation and purine ribonucleotide metabolism. Mol Pharmacol 41, 671–676 (1992)
17. Mosmann T: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 65, 55–63 (1983)
18 Cunningham AJ and Szenberg A: Further improvements in the plaque technique for detecting single antibody forming cells. Immunology 14, 599–600 (1968)

19 Sidi Y and Mitchell BS: 2'-Deoxyguanosine toxicity for B and mature T lymphoid cell lines is mediated by guanine ribonucleotide accumulation. J Clin Invest 74, 1640–1648 (1984)

20 Kazmers I, Mitchell BS, Daddona P, Wotring L, Townsend L and Kelley WN: Inhibition of purine nucleoside phosphorylase by 8-aminoguanosine: Selective toxicity for T lymphoblasts. Science 214, 1137–1139 (1981)

21 Okubo M, Chen X-M, Kamata K, Masaki Y and Uchiyama T: Immunosuppressive effect and mode of action of cyclosporin A (CyA) and mizoribine (MZR) on humoral immune response in mice. Jpn J Nephrol 28, 51–59 (1986) (Abstr in English)

22 Terai C, Hakoda M, Yamanaka H, Kamatani N and Kashiwazaki S: Differential cytotoxic effects of mizoribine and its aglycone on human and murine cells and on normal and enzyme-deficient human cells. Biochem Pharmacol 50, 1099–1102 (1995)

23 Hirohata S and Yanagida T: Inhibition of expression of cyclin A in human B cells by an immunosuppressant mizoribine. J Immunol 155, 5175–5183 (1995)