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Differential modulation of IL-2- and IL-4-mediated early activation of normal human B lymphocytes by the caspase inhibitor zVAD-fmk



Abstract

Caspases are a group of cysteine-related proteases that control the process of apoptosis and may also be involved in the control of lymphocyte activation. We show here that the broad-spectrum caspase inhibitor benzyloxycarbonyl (Cbz)-Val-Ala-Asp (Ome)-fluoromethylketone (zVAD-fmk) prevents the proliferation of resting human B tonsillar lymphocytes mediated by the B cell mitogen SAC or the

combination of anti-m Ab and IL-2. zVAD-fmk inhibits IL-2-induced phosphorylation of the retinoblastoma protein, and cyclin D2 expression. However, neither the IL-2-mediated proliferation of cycling activated B cells nor that of lymphoma cells were inhibited by zVAD-fmk, suggesting that only the early steps of SAC- or IL-2-mediated B cell activation were sensitive to the inhibitory properties of zVAD-fmk. Our data also demonstrated that the inhibitory effect of zVAD-fmk was not observed when B cells were activated with IL-4 in the presence of either anti-m Ab or anti-CD40 Ab. Thus, our results suggest that caspase activation is required for the IL-2-mediated entry of primary B lymphocytes into the cell cycle and show that caspase activation plays different roles in IL-2- and IL-4-mediated B cell proliferation.

Full text

Introduction

The engagement of B cell antigen (BCR) and T cell antigen (TCR) receptors induces the proliferation of B and T cells respectively. Lymphocyte activation is a highly regulated process involving various intracellular signaling pathways and a large number of molecules. It has recently been suggested that caspases, a family of cysteine proteases that play a crucial role in controlling the executionary phases of apoptosis, may be involved in the control of lymphocyte proliferation [1-3]. These caspases are synthesized as inactive proenzyme, and they are activated by proteolysis at specific aspartate sites. The resulting cleavage products form dimers that are the active enzymes. Caspases are defined as initiator, effector and inflammatory proteases, the activity of which can be modulated by synthetic peptide inhibitors [4-10].

Recent observations have suggested that, in addition to their role in controlling apoptosis, caspases may also be involved in the control of T lymphocyte activation. Indeed, interference with the pathway leading to caspase activation, as in FADD knock-out mice and the FADD dominant negative form, results in retardation of cell cycle progression in primary T cells [11-13]. More directly, two different groups have recently reported that synthetic caspase inhibitors also prevented T cell activation in response to anti-CD3 Ab and IL-2 [2, 3]. The proliferation of resting human B cells may be triggered, in the presence of anti-m Ab, by IL-2 or IL-4. We therefore investigated whether either IL-2- and/or IL-4-mediated B cell activation was also dependent on caspase activation.

We have demonstrated that *in vitro* treatment with the broad-spectrum, permeable caspase inhibitor zVAD-fmk, prevents IL-2-mediated, early B cell activation of resting tonsillar B cells leading to cell cycle entry. However, no such inhibition was observed if B cells were activated in the presence of IL-4. These data suggested that caspase activation has different roles in IL-2 and IL-4-induced B cell proliferation.

Methods

Reagents

Recombinant IL-2 was provided by Chiron Laboratory-France (Suresnes, France). Recombinant IL-4 was purchased from Genzyme (Boston, MA, USA). SAC, *Staphylococcus aureus* Cowan 1 strain (pansorbin) and non-solubilized rabbit anti-m antibodies were obtained from Calbiochem (France Biochem) and Biorad (Richmond, CA, USA) respectively. z-Val-Ala-DL-Asp-fluoromethylketone (zVAD-fmk) was supplied by Bachem Biochimie SARL (Voisin le Bretonneux, France).

Cell preparations

Normal B cells were isolated from human tonsils as previously described [14]. Briefly, single-cell suspensions were depleted of T-cells by two cycles of rosetting with 2-aminoethyl-isothiuronium-treated sheep red blood cells and depleted of monocytes by adhesion onto plastic. B cells were then separated into density fractions on discontinuous Percoll gradients (Pharmacia Fine Chemicals, Uppsala, Sweden). Cells suspended below the 60% Percoll fraction (a homogeneous population of small cells, $> 97 \text{ IgD}^+/\text{CD38}^-$ cells) were considered to be resting cells. Ramos cells were obtained from the ATTC (Rockville, MD, USA) and the human T-cell chronic lymphocytic leukemia-derived, IL-2 dependent Kit 225 cell line was kindly provided by Dr. Kori (Kyoto University, Japan).

B cell cultures

B cells ($10^6/\text{ml}$) were cultured in RPMI 1640 culture medium (Gibco, Grand Island, NY, USA) supplemented with 1% glutamine, 1% antibiotics, 2-bME ($0.5 \times 10^{-5} \text{ M}$) and 10% fetal bovine serum (Gibco), in the presence of either SAC (1/10000) or anti-m Ab (10 mg/ml) with or without IL-2 (100 U/ml) or IL-4 (100 U/ml) for

72 hours. For the experiment presented in Figure 4A, B cells (10^6 /ml) were activated before the experiment with SAC (1/10 000) for 3 days in tissue culture flasks (Corning, CA, USA). The activated B cells were recovered, washed, counted, and the effect of IL-2 was determined as described above. The proliferative response was measured by addition of 0.5 mCi of (^{3} H)TdR (CEA, Saclay, France) for the last 16 h of 3-day cultures. RNA synthesis was measured by addition of 1 mCi of (^{3} H)uridine (CEA) for the last 6 h of 24-hour cultures.

Cell size analysis

B cell activation was detected by cytometric analysis of dot-blot light scatter profiles. The cells (10^6) were washed in PBS and resuspended in 1% PFA (paraformaldehyde) in PBS. After 1 h of incubation at room temperature, activated cells were analyzed for their dot-blot light scatter profile by flow cytometry using a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA, USA). Activated cells having relatively low side-scatter and high light forward-scatter properties were counted and their number expressed as a percentage of the total population.

Western blotting

Cells were lysed by incubation in lysis buffer (50 mM Tris pH 8, 150 mM NaCl, 0.1% Nonidet P40, 5 mM EDTA, 50 mM NaF, 2 mM sodium vanadate, 1 mg/ml aprotinin, 2 mM PMSF, 1 mM DTT, 10 mg/ml leupeptin, 2 mg/ml pepstatin A) (all from Sigma,) on ice for 60 min. The lysate was centrifuged for 30 min at 13,000 g at 4°C and the supernatant assayed for protein concentration (micro-BCA protein assay, Pierce chemical Co., Rockford, IL, USA). Cell lysate proteins (50 mg) were boiled for 5 min in 2X sample buffer and resolved on 10 or 13% SDS polyacrylamide gels by electrophoresis. Proteins were electroblotted onto 0.45 m nitrocellulose filters and the filters blocked for 1 hour at room temperature with 5% non-fat milk in PBS, 1% Tween 20. Filters were then incubated for 1 hour with anti-cyclin D2 (2 mg/ml) (Santa Cruz Inc, Santa Cruz, CA, USA), anti-pRb (2 mg/ml) (clone G3-245) (Pharmingen, San Diego, CA, USA) or anti-actin (1 mg/ml). The blots were washed three times (10 min) with 0.1% Tween 20 in PBS and incubated (60 min) with peroxidase-labeled swine anti-rabbit or sheep anti-mouse immunoglobulin (1/5000) (Amersham, Les Ulis, France). Blots were developed using an enhanced chemiluminescence detection system (ECL, Amersham). Films were exposed for 1 to 30 min.

Results

We investigated the involvement of caspases during human B cell activation by first determining the ability of the permeable broad-range caspase inhibitor zVAD-fmk to regulate B lymphocyte proliferation. Resting human B lymphocytes were cultured with the B cell mitogen *Staphylococcus aureus* Cowan 1 strain (SAC), in the presence or absence of various concentrations of zVAD-fmk, and B cell proliferation was determined by measuring thymidine uptake over the last 16 hours of a 3-day culture. As shown in Figure 1A, SAC-induced thymidine uptake was inhibited by 10 mM zVAD-fmk, and inhibition was maximal at a concentration of 100 mM. In contrast, similar amounts of the carrier, DMSO, had no effect on thymidine uptake. We next investigated whether a more physiological stimulus, based on the combined B cell activation through the BCR in the presence of IL-2 was also sensitive to the inhibitory effect of zVAD-fmk. Indeed, as shown in Figure 1B, thymidine uptake observed upon costimulation with anti-m Ab and IL-2 was diminished in the presence of 60 mM zVAD-fmk; inhibition was maximal with 120 mM zVAD-fmk whereas no inhibition was observed in the presence of DMSO alone, used as a control.

To determine which step of B cell activation was sensitive to the inhibitory effect of zVAD-fmk, we next investigated whether earlier steps, including RNA synthesis and cell size enlargement, were also inhibited by this broad-spectrum synthetic caspase inhibitor. As shown in the Figure 2A, uridine uptake, measured after 20h of stimulation with anti-m antibody and IL-2, was also inhibited in the presence of 60-120 mM concentrations of zVAD-fmk. This inhibitory effect of zVAD-fmk was correlated with an important inhibition of cell size enlargement observed upon anti-m antibody and IL-2 activation (24% in the presence of zVAD-fmk versus 63% in activated control cells and 3% in non-activated cells) (Figure 2B).

Cell cycle entry and early G1 progression are tightly controlled by the activity of cell cycle specific serine/threonine kinases (cdk), the activity of which is dependent on expression and association with the proteins of cyclin D. Among their different substrates, the cdk-dependent phosphorylation of retinoblastoma protein (Rb) is a milestone of G1-progression through the restriction point R1. We have previously reported that, in human B cells, such Rb phosphorylation depends on the sequential expression of cyclin D2 and cyclin D3 [15]. We therefore investigated whether zVAD-fmk interfered with both the

cyclin D2 expression and Rb phosphorylation induced by anti-m Ab and IL-2 (Figure 3). As previously reported, resting B cells do not express either cyclin D2 or Rb protein [15]. After 48h of activation with anti-m Ab and IL-2, cyclin D2 was detected in activated cells and its production was found to be associated with both the production and the phosphorylation of Rb, as shown by the appearance of an additional band, corresponding to different degrees of phosphorylation of this protein. In the presence of zVAD-fmk (100 mM), i) considerably less cyclin D2 was present in activated cells and ii) expression of the upper band of Rb, corresponding to the phosphorylated form (pRb) was totally inhibited whereas the global level of Rb expression was not modified. Altogether, these data are consistent with zVAD-fmk preventing the entry into the cell cycle of human B cells activated with anti-m Ab and IL-2.

We then investigated the effect of zVAD-fmk on various types of cycling cells, including pre-activated tonsilar B cells, B and T lymphoma cells depending or not on IL-2 for their growth. We first studied the response to IL-2, of 3 day SAC-preactivated tonsilar B cells (Figure 4A). As previously reported, these cells, which appears as activated large cells, did not proliferate after 3 days of culture. However, they did proliferate later in the presence of IL-2. This proliferation was monitored by assessing thymidine uptake during the last 16h of a secondary 3 day culture, in the presence of IL-2 [16]. In these conditions, z-VAD-fmk, even at a concentration of 120 mM, did not decrease the incorporation of thymidine, suggesting that the response of these preactivated cells to IL-2 was caspase-independent. A similar lack of effect of zVAD-fmk was also observed during the proliferation of the Ramos human Burkitt lymphoma B cells (Figure 4B). We also investigated the effect of zVAD-fmk on the proliferative response of the IL-2-dependent T lymphoma cells Kit 225 [17]. As shown in Figure 4C, zVAD-fmk did not block the proliferative response of Kit 225 cells to IL-2. Thus, all these data are consistent with our hypothesis that zVAD-fmk inhibits entry into the cell cycle of resting human B cells in response to SAC or IL-2.

Cytokines other than IL-2, such as IL-4, can also promote entry into the cell cycle of resting human B cells [15]. We therefore investigated whether the proliferation of resting tonsilar B cells, activated with anti-m Ab and IL-4, was also influenced by zVAD-fmk. We found that zVAD-fmk had no effect on the proliferative response to anti-m and IL-4. In contrast, 120 mM zVAD-fmk almost completely abrogated the proliferative response of these cells to IL-2 (Figure 5A). To confirm this observation, we next checked that the activation of resting

B cells with IL-4 in association with other costimulatory stimuli, such as anti-CD40 Ab, was also resistant to the inhibitory effects of zVAD-fmk. Indeed, the data presented in the Figure 5B, resulting from data obtained from 6 different experiments, clearly showed a clear difference in the effects of zVAD-fmk on IL-2- and IL-4-mediated B cell proliferation. Whereas IL-2- mediated proliferation was greatly reduced by 120 mM zVAD-fmk, similar concentrations of the caspase inhibitor did not strongly inhibit the IL-4-mediated proliferation observed in the presence of anti-m Ab, anti-CD40 Ab or the combination of both of the two stimuli.

Discussion

We demonstrate here that zVAD-fmk, a broad-spectrum caspase inhibitor, prevents the activation of resting B cells in response to IL-2, suggesting that cysteine proteases are directly involved during the early phases of B cell activation. The nature of the caspases mobilized during this process in B cells is not clear. In T cells, it has been reported by two different groups that caspase-8 is directly involved in the control of the early steps of T cell activation [2, 3]. Nevertheless, although we were able to clearly detect caspase-8 activation during the apoptotic process mediated by either TGFb or cross-linked anti-m Ab in B lymphoma cells [9, 10], we did not observed caspase-8 activation in IL-2- or SAC- activated resting B cells. None of the other so-called regulatory caspases, such as caspase-2 or caspase-10 were detected in an active form in these cells (data not shown). Similarly, as reported by Alam et al., we observed caspase-3 activation in both SAC- and IL-2-activated B cell (2 and data not shown). However, the cleaved forms of caspase-3 appeared only after 3-4 days of culture. These kinetics are not compatible with the early effects of zVAD-fmk reported in this paper. Using different synthetic peptide inhibitors, specific for individual caspases (DEVD-fmk for caspase-3 and 6, 7, 8 and 10, IETD-fmk and AEVD-fmk for caspase-8 and 10, VDVAD-fmk for caspase-2, VEID-fmk for caspase-6 and LEHD-fmk for caspase-9), we could not clearly target the caspase(s) responsible for this effect. Thus, either the different tools available are not sufficiently accurate to identify this caspase or the caspase involved during the process of early B cell activation is a new, as yet uncharacterized cysteine protease.

ZVAD-fmk-mediated inhibition occurs at a very early step in activation that could be related, by different criteria, to the entry of these cells into the cell cycle.

Indeed, zVAD-fmk blocks the cell enlargement and the phosphorylation of Rb associated with G1 progression. More directly, expression of cyclin D2, which take place in early G1, was deeply diminished in the presence of this caspase inhibitor. The demonstration that the inhibitory activity of zVAD-fmk occurs during the early steps of B cell activation is strengthened by the observations that the proliferative response to IL-2 of cycling, activated cells, including normal B cells and lymphoma T cells, was not inhibited by zVAD-fmk.

The molecular bases of this inhibition are not clear. Various effectors of cell cycle regulation are potential target for caspases. Indeed, we and others have previously reported that Rb protein may be cleaved during apoptosis, and the resulting cleaved form of Rb may be partly responsible for the apoptotic stimulus [18, 19]. However, the involvement of such Rb cleavage can be excluded in our experimental conditions since 1) this cleaved fragment was not detected in IL-2-activated B cells and 2) this cleavage is mediated by caspase-3 [19], which is not activated during the time course of our culture, and 3) using different criteria, we never observed zVAD-fmk-mediated increases in apoptosis in SAC or anti-m Ab/ IL-2 activated B cells (data not shown). Among other important regulators of the cell cycle, inhibitors of cdks, such as p21 has also been reported to be targets for caspase-3 [20]. Indeed, we observed some degree of p21 degradation in anti-m/IL-2-activated B cells, but only after 48 h of culture when the addition of zVAD-fmk no longer prevented IL-2-induced proliferation (data not shown). Thus, it seems unlikely that the inhibitory activity of zVAD-fmk is associated with the caspase-mediated cleavage of molecules regulating the cell cycle.

Interestingly, we found that zVAD-fmk inhibited cyclin D2 expression. This observation suggests that zVAD-fmk could act upstream of cyclin D2 transcription. Thus, in this case, zVAD-fmk-mediated inhibition would be associated with the caspase-dependent regulation of transduction pathways regulating the expression of various proteins including cyclin D2. In fact, IkbB has been reported to be a possible target of caspases [21, 22]. Under our experimental conditions, we obtained no evidence for the cleavage of IkbB, suggesting that this molecule is not involved in the zVAD-fmk-mediated-inhibition of B cell activation. Experiments are underway in our laboratory to characterize the molecules, the expression or activity of which is sensitive to zVAD-fmk in the context of anti-m/IL-2 activation leading to B cell proliferation.

B cell proliferation observed in the presence of anti-m Ab or CD40 Ab was less sensitive to zVAD-fmk inhibition. There are different explanation for this observation: i) these stimuli activated different subset of B cells, ii) the transduction pathways activated by these stimuli display differential sensitivity to caspases and/or iii) a combination of these two hypotheses. The nature of the transduction pathways activated by SAC is still unclear. Although there is no direct demonstration, we could speculate that SAC, like LPS in the mouse, may activate B cells through a Toll family receptor, the transduction pathway of which may include effectors sensitive to caspase-activity [23]. In contrast to IL-2, IL-4 can directly activate resting B cells, inducing an increase in the production of different molecules, including transcription factors such as c-myc, and surface molecules as CD23 [24, 25]. Thus it is possible that IL-4 activates caspase-independent effectors, not present in IL-2 signaling, which would cooperate with anti-m Ab and CD40 signaling to promote B cell proliferation.

Our data show that the broad-range caspase inhibitor zVAD-fmk inhibits early B cell activation and cell cycle entry, depending on the presence of certain activating stimuli. The mitogenic signal provided by IL-4 was independent of caspase activation, whereas IL-2-mediated B cell activation was highly sensitive to zVAD-fmk. Thus, a strategy based on the use of caspase inhibitors to prevent *in vivo* apoptosis may have underestimated the side effects involving the immune response. Finally, our data support the emerging notion that caspase activation is not restricted to the apoptotic process, but could be directly involved during the activation of primary resting lymphocytes. We observed that the inhibitory effects of zVAD-fmk depended on the nature of the stimuli responsible for B cell proliferation. Thus, our results suggest that caspase activation is required for the IL-2-mediated entry of primary B lymphocytes into the cell cycle, and show that caspase activation has different roles in IL-2 and IL-4-mediated B cell proliferation.

CONCLUSION

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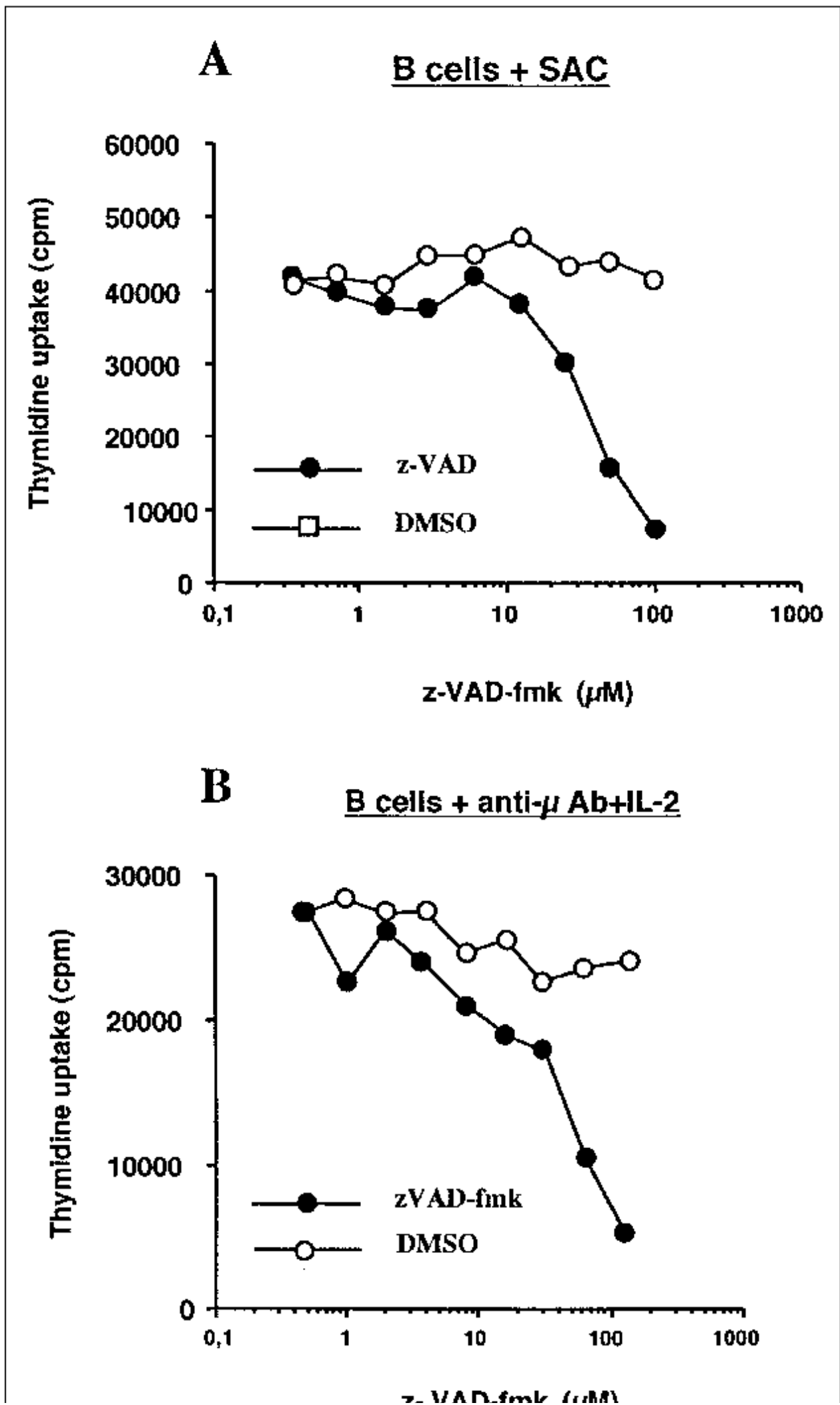
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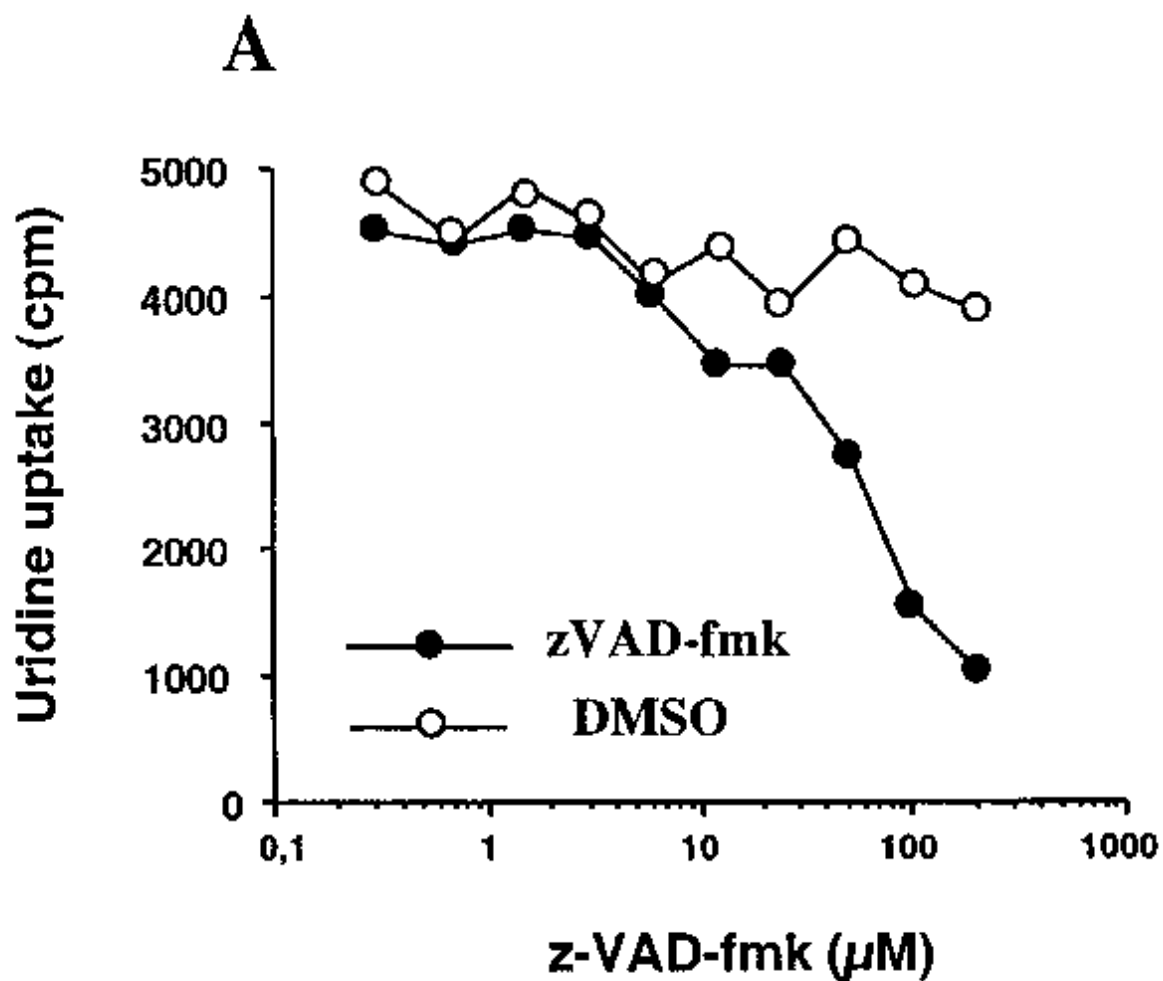
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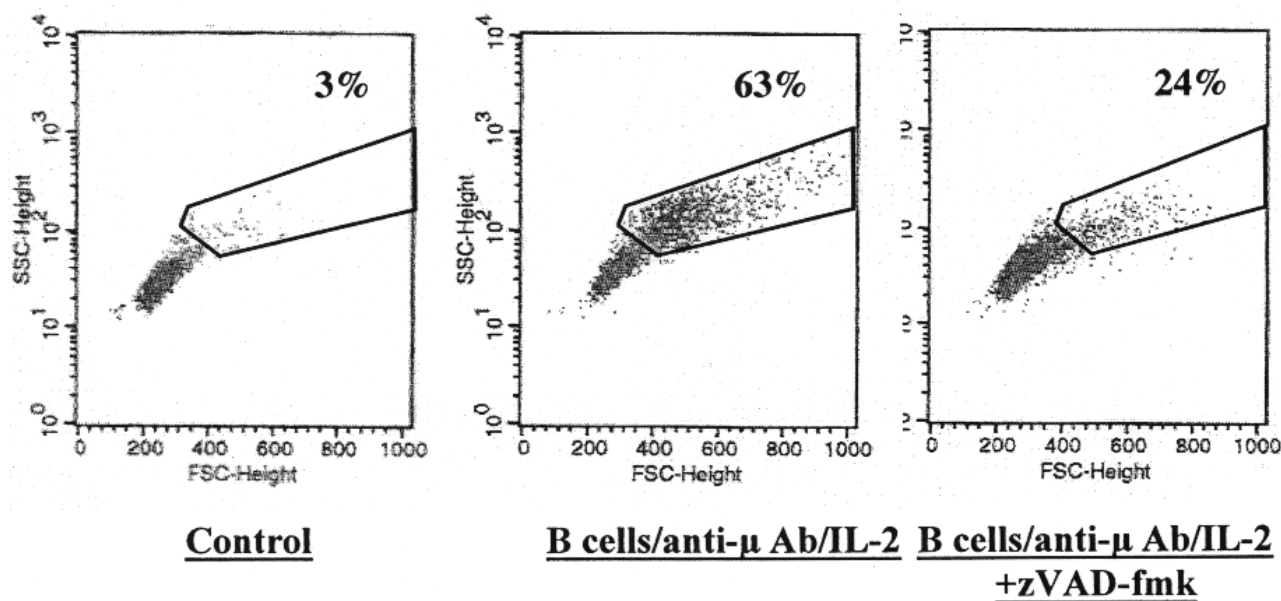
Figures



ZVAD-fmk inhibits SAC and IL-2-mediated B cell proliferation.

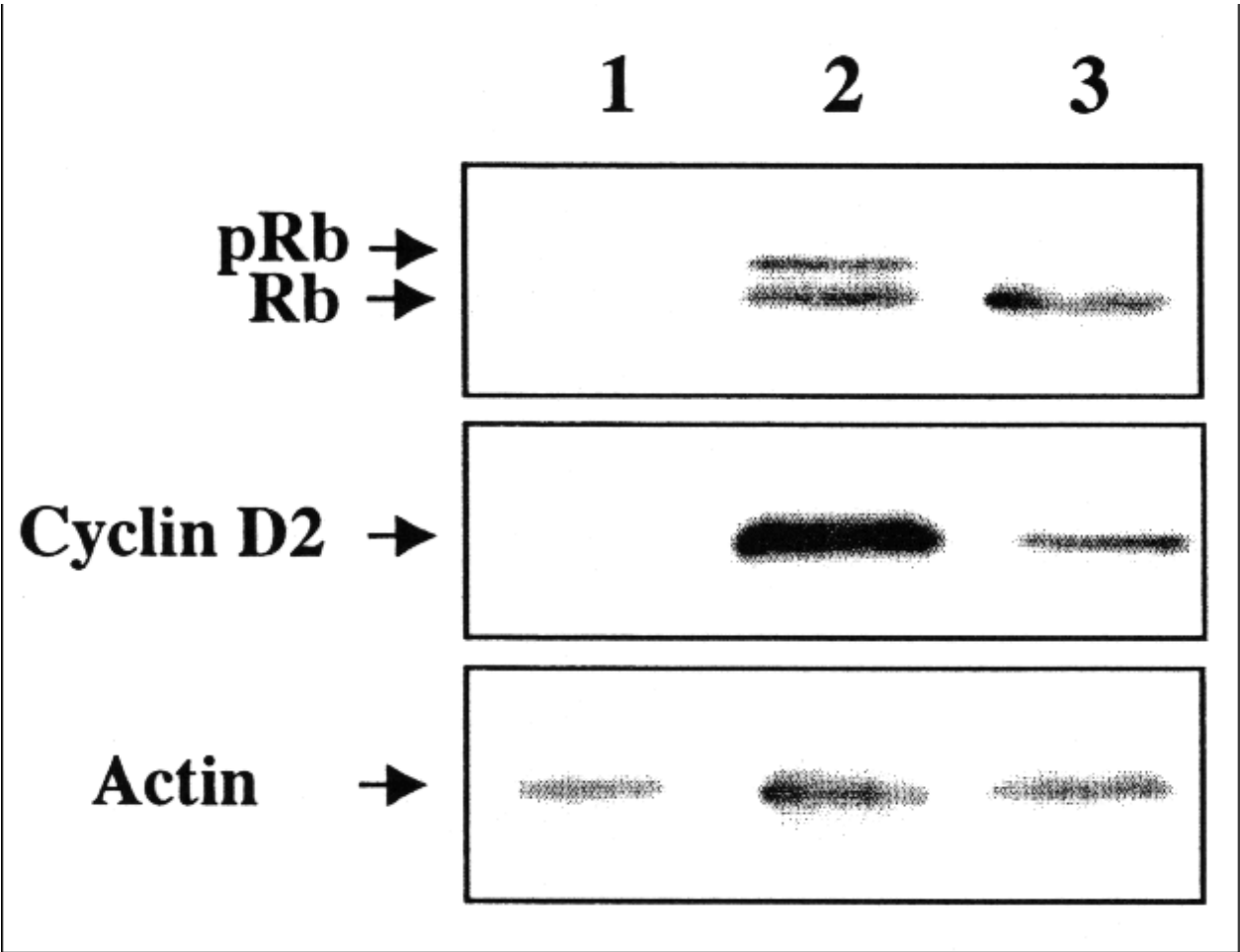
Highly purified human B cells (10^6 /ml) were activated by incubation for 72 h in the presence of SAC (1/10 000 final dilution) (Figure 1A) or a combination of anti-m Ab (10 mg/ml) and IL-2 (100 U/ml) (Figure 1B) with various concentrations of zVAD-fmk or with vehicle (DMSO) as the control. DNA synthesis was quantified by measuring ^3H -thymidine incorporation during the last 16 h of culture.



B

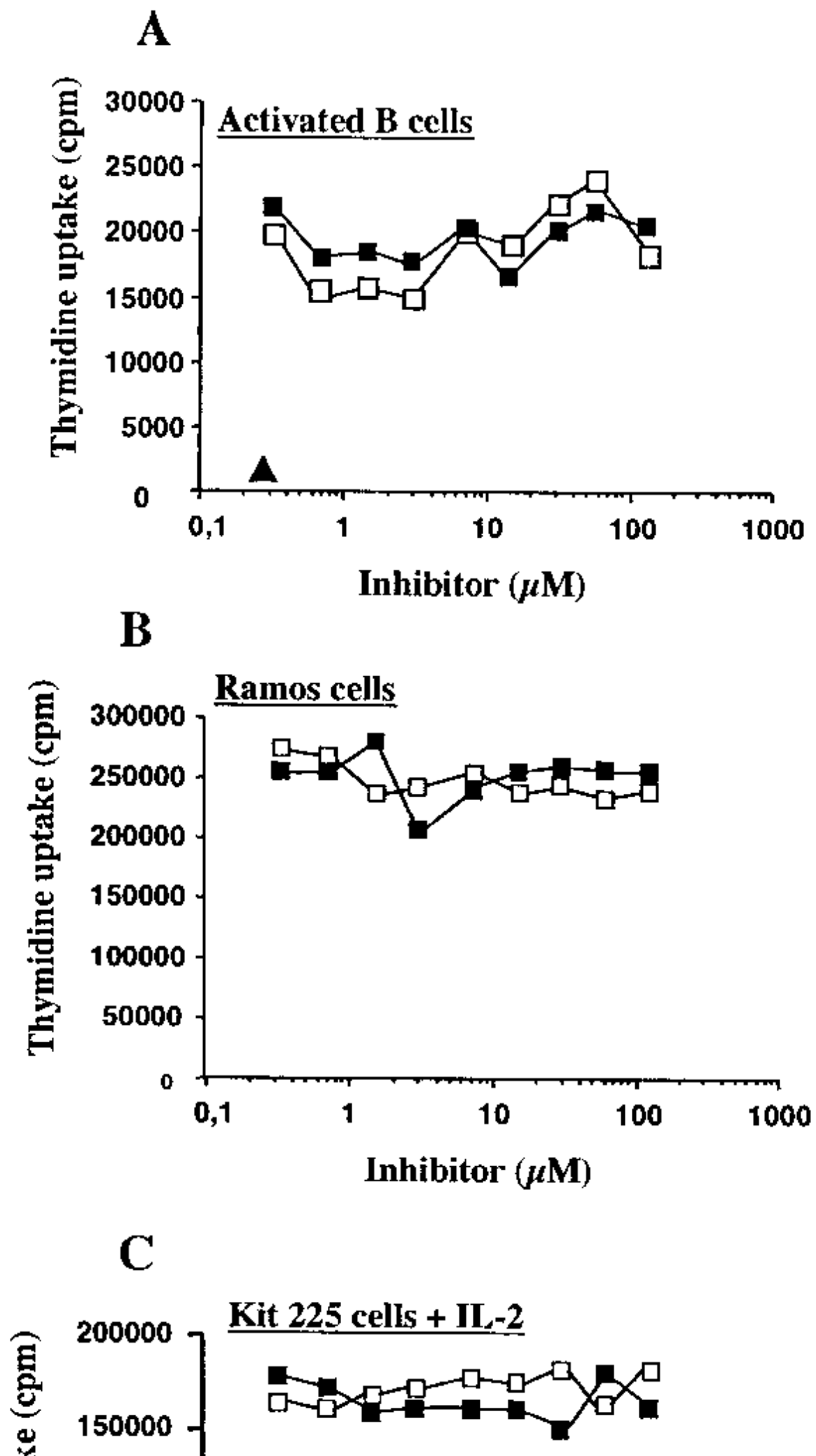
zVAD-fmk inhibits early steps of IL-mediated B cell activation.

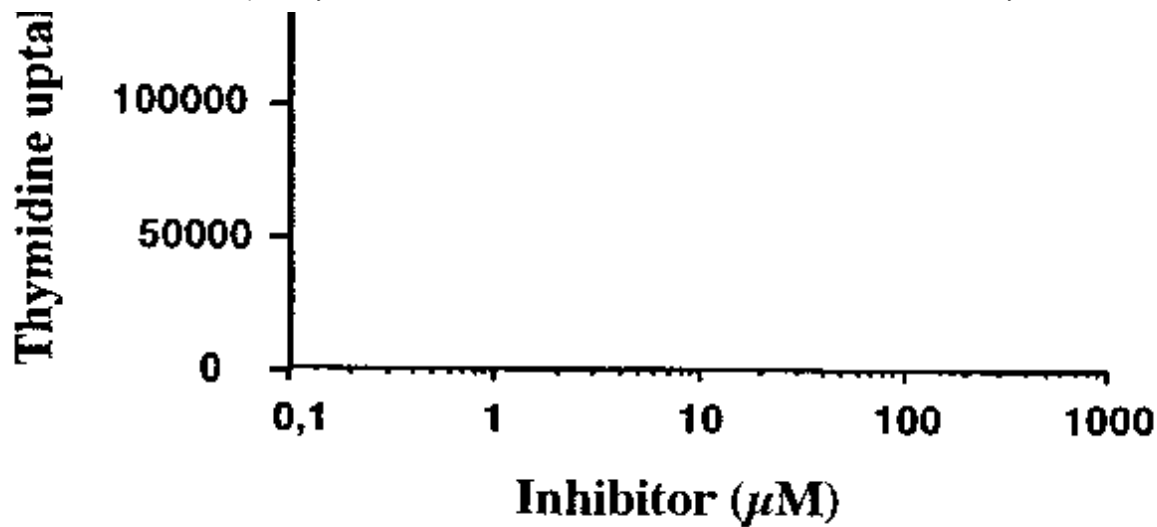
Highly purified human B cells (10^6 /ml) were activated for 36 h in the presence of a combination of anti-m Ab (10 mg/ml) and IL-2 (100 U/ml) (Figure 2A) with various concentrations of zVAD-fmk or with control DMSO. RNA synthesis was quantified by measuring ^{3} H-uridine incorporation during the last 16 h of culture. For Figure 2B, cells were stimulated with anti-m Ab and IL-2 for 24h in the absence or presence of 100 mM zVAD-fmk, and enlarged activated cells with increased forward-scatter (FSC) were quantified by flow cytometry.



zVAD-fmk inhibits IL-2-induced cyclin D2 expression and Rb phosphorylation.

Cell lysates (50 mg) of resting B cells (lane 1), or B cells activated for 48 h with a combination of anti-m Ab (10 mg/ml) and IL-2 (100 U/ml) in the absence (lane 2) or the presence of 100 mM zVAD-fmk (lane 3) were analyzed for Rb phosphorylation by immunoblotting using anti-Rb Ab. The same membrane was sequentially stripped and then reprobed with anti- cyclin D2 Ab. The amount of protein loaded onto each lane was assessed by probing the filter with a specific Ab for human actin.



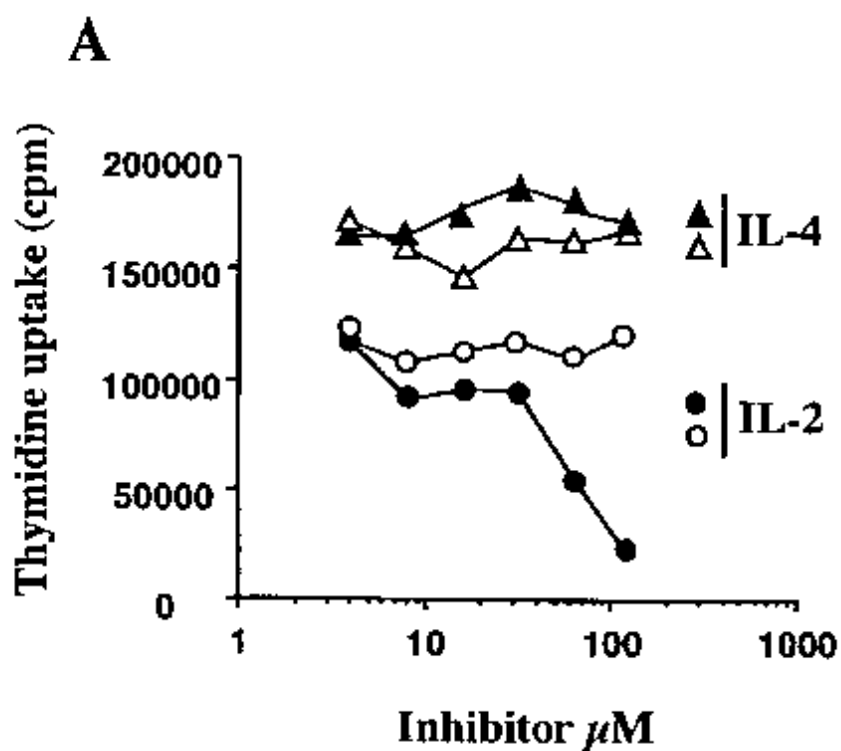
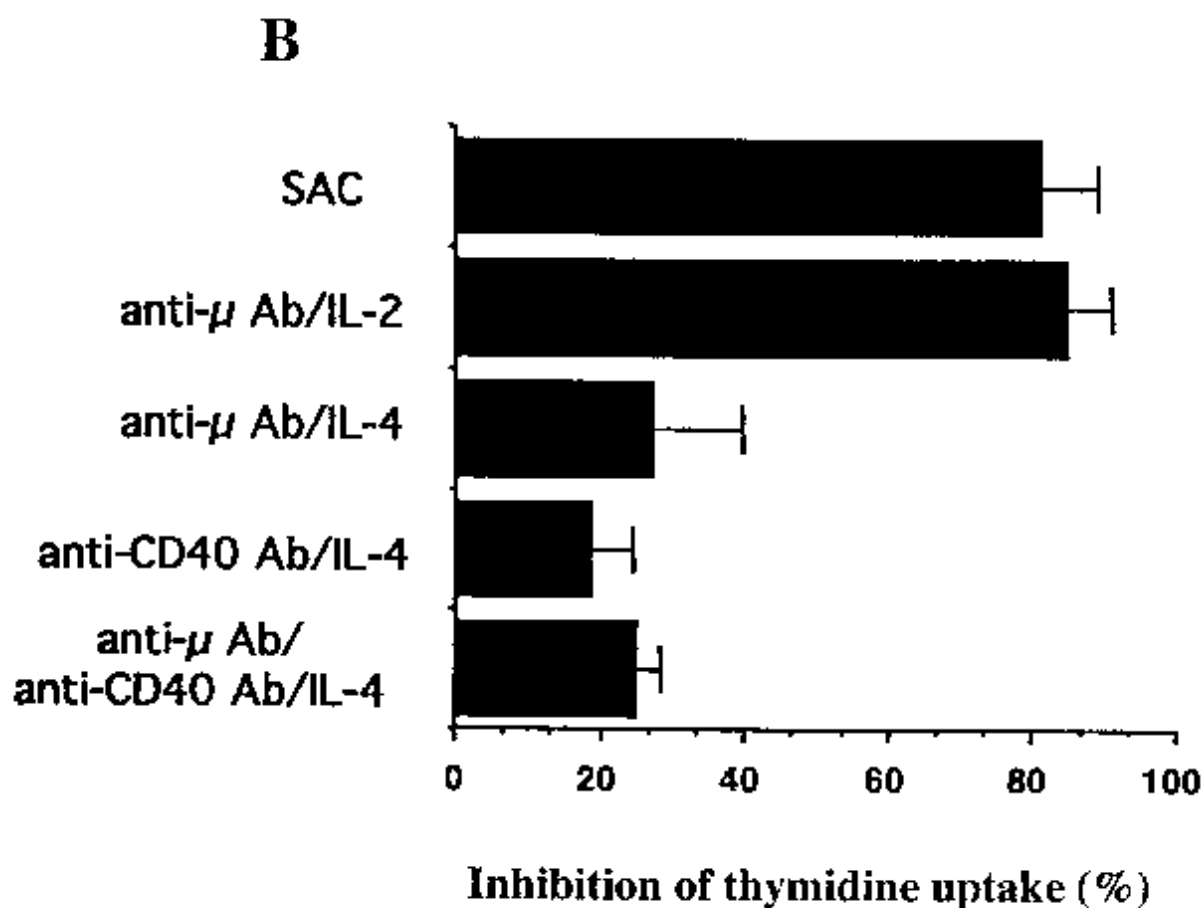


zVAD-fmk did not inhibit the proliferation of activated lymphocytes.

Figure 4A. Purified B cells were activated with SAC (1/10 000) for 3 days. Activated B cells were further activated without (closed triangle) or with IL-2 (100 U/ml) in the presence of various doses of zVAD-fmk (closed square) or control DMSO (open square). DNA synthesis was quantified by measuring ^3H -thymidine incorporation during the last 16 h of a 3-day culture.

Figure 4B: Ramos Burkitt B cells ($3 \cdot 10^5/\text{ml}$) were cultured for 48h in the presence of various doses of zVAD-fmk (closed square) or control DMSO (open square). DNA synthesis was quantified by measuring ^3H -thymidine incorporation during the last 16 h of a 2-day culture.

Figure 4C: IL-2-dependent Kit 225 T cells were synchronized by culture in the absence of IL-2 for 72 h. Cells were activated with IL-2 (100 U/ml) for 48h. In the presence of various doses of zVAD-fmk (closed square) or control DMSO (open square). DNA synthesis was quantified by measuring ^3H -thymidine incorporation during the last 16 h of a 2-day culture.



In contrast to IL-2, IL-4-mediated B cell activation is not inhibited by zVAD-fmk.

Figure 5A: Highly purified human B cells (10^6 /ml) were activated for 72 h in the presence of a combination of anti-m Ab (10 mg/ml) and IL-2 (100 U/ml) (circles) or IL-4 (triangles) with graded concentrations of zVAD-fmk (closed symbols) or control DMSO (open symbols). DNA synthesis was quantified by measuring ^3H -thymidine incorporation during the last 16 h of culture.

Figure 5B. Highly purified human B cells (10^6 /ml) were activated for 72 h in the presence of SAC (1/10 000), or a combination of anti-m Ab (10 mg/ml), anti-CD40 Ab (10 mg/ml), IL-2 (100 U/ml) or IL-4 (100 U/ml). Data are the mean of 5 different experiments.

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liver failure, apoptosis, tumor necrosis factor, NF-KB, interleukin-6, Fas

○ Presented at the 4th symposium on Cytokines and apoptosis in the cardiovascular system, Halle, Germany,

June 6-7, 2002

○ Activated STAT-1 pathway in the myocardium as a novel therapeutic target in ischaemia/reperfusion injury

Anastasis Stephanou

STAT, heart, ischaemia/reperfusion, apoptosis

○ A subset of caspase substrates functions as the Jekyll and Hyde of apoptosis

Jian-Yan Yang, Christian Widmann

RasGAP, MEKK1, caspase substrates, apoptosis, survival

○ Compromised IFN-gamma production in the elderly leads to both acute and latent viral antigen stimulation: contribution to the immune risk phenotype?

Qin Ouyang, Wolfgang M Wagner, Anders Wikby, Ed Remarque, Graham Pawelec

Aging, T-cell, IFN-g, CMV, EBV, immune risk phenotype, longitudinal study

○ Cytokines in chronic heart failure: possible involvement in the neurohormonal and the cytokine systems at the cAMP level?

Claudia Werner, Harald Loppnow, Mathias Rauchhaus, Armin Wessel, Karl Werdan, Reiner Buchhorn

Adult, b-blocker, children, chronic heart failure, cyclic AMP, cytokine, infant, interleukin, soluble TNF receptor, tumor necrosis factor

○ Effects of tumor necrosis factor-alpha and interleukin-6 in elderly populations

Helle Bruunsgaard

TNF-a, IL-6, aging, mortality, inflammation

○ Energetic depression caused by mitochondrial dysfunction

Frank Norbert Gellerich, Sonata Trumbeckaite, Ying Chen, Marcus Deschauer, Tobias Müller, Stephan Zierz

Mitochondria, outer membrane, inner membrane, mitochondrial disease, sepsis, phosphorylation potential

○ Senescence: the cost of being young and healthy?

Graham Pawelec

○ The regulation of death-associated protein (DAP) kinase in apoptosis

Galit Shohat, Taly Spivak-Kroizman, Miriam Eisenstein, Adi Kimchi
apoptosis, DAPk, autophosphorylation, negative regulation

○ Neisseria meningitidis can induce pro-inflammatory cytokine production via pathways independent from CD14 and toll-like receptor 4

Tom Sprong, Peter van der Ley, Liana Steeghs, Will J Taw, Trees JW Verver-Janssen, Mihai G Netea, Jos WM van der Meer, Marcel van Deuren
Neisseria meningitidis, lipopolysaccharide, endotoxin, cytokines, CD14, TLR4

○ Evaluation of tumor necrosis factor alpha production in ex vivo short term culture whole blood from women with polycystic ovary syndrome

A Veronica Araya, Adam Aguirre, Carmen Romero, Cristian Miranda, Maria Carmen Molina, Arturo Ferreira
LPS, polycystic ovary syndrome, TNF- α , whole blood cell culture

○ Plasma levels of IL-12, IL-18 and TGF-beta in Plasmodium falciparum malaria

Lucia Malaguarnera, Salvatore Pignatelli, Jacques Simporè, Mariano Malaguarnera and Salvatore Musumeco
IL-12, IL-18, TGF- β , Plasmodium falciparum

○ In vitro analysis of IGn-gamma and IL-12 production and their effects in ileal Crohn's disease

Stefaan Colpaert, Kathleen Vastraelen, Zhanju Liu, Philippe Maerten, Chong Shen, Freddy Penninckx, Karel Geboes, Paul Rutgeerts, Jan L Ceuppens
IL-12, IFN- γ , anti-IL-12/ anti-IFN- γ , in vitro, Crohn's disease

○ Differential modulation of IL-2- and IL-4-mediated early activation of normal human B lymphocytes by the caspase inhibitor zVAD-fmk

Shahul Mouhamad, Damien Arnoult, Marie-Thérèse Auffredou, Jérôme Estaquier, Aimé Vazquez

IL-2, IL-4, B lymphocyte, caspase, proliferation, cell cycle entry

○ Prolactin-cytokine network in the defence against *Acanthamoeba castellani* in murine microglia

Nunzia Benedetto, Claude Auriault
Prolactin, cytokine, *A. castellani*, MGC.

○ CINC-1 mediates the sympathetic component of inflammatory mechanical hypersensitivity in rats

Berenice B Lorenzetti, Fabiane H Veiga, Claudio A Canetti, Stephen Poole, Fernando Q Cunha, Sergio H Ferreira
Inflammatory hyperalgesia, CINC-1, tumour necrosis factor alpha, interleukin-1b, interleukin-8, sympathetic mediators, prostaglandin E2

○ A CXC chemokine sequence isolated from the rainbow trout *Oncorhynchus mykiss* resembles the closely related interferon-gamma-inducible chemokines CXCL9, CXCL10 and CXCL11

Kerry J Laing, Niels Bols, Christopher J Secombes
Chemokine, rainbow trout, IP10, I-TAC, Mig

○ Meeting report of the “Club Cytokine” (French Society of Immunology) meeting, in Le Croisic, France

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