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Participation of tyrosine kinase in capping, internalization, and antigen presentation through membrane immunoglobulin in BAL17 B lymphoma cells

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BAL17 cells pulsed with goat anti-IgM or anti-IgD as antigens stimulated a goat IgG specific T cell clone in terms of inositol phosphate production. The antigen-presenting capacity of BAL17 cells was inhibited by pretreatment with the tyrosine kinase inhibitors herbimycin A or genistein. Furthermore, ligand-induced capping and endocytosis of membrane immunoglobulin, monitored at the single cell level, was also blocked by herbimycin A. These results indicate that tyrosine phosphorylation plays an important role in receptor-mediated antigen presentation by B cells.

B cell; Capping; Membrane immunoglobulin; Antigen presentation; Tyrosine kinase; Internalization

1. INTRODUCTION

B cells take up antigens via pinocytosis or receptor-mediated events, one of which is an immunoglobulin (mIg)-induced step. The internalized antigen is processed and presented to T cells, which then become activated [1,2]. The mIg-mediated uptake of an antigen is far more efficient than pinocytosis and is considered to play an essential role in antibody response through a collaboration between B cells and T cells [1,2].

Ligation of mIg stimulates B cells to activate tyrosine phosphorylation of several proteins including phospholipase C (PLC) [3–6]. The activation of PLC has been shown to be associated with the activation of the enzyme leading to generation of inositol triphosphate and diacylglycerol through phosphatidylinositol breakdown [5–7]. Inositol triphosphate increases the levels of intracellular calcium and diacylglycerol activates protein kinase C [7,8]. B cells contain at least three species of tyrosine kinase, namely fyn, lyn, and blk, which are associated with mIgM or mIgD [9–12]. These kinases are phosphorylated at a tyrosine residue after mIg cross-linking, which probably correlates with the activation of the enzyme and controls the critical early events in B cell activation [11–14]. Using tyrosine kinase inhibitors, we examine the effect of tyrosine kinase on the capacity of B cells to present the antigen taken up through mIg to T cells. We show that tyrosine kinase participates in antigen-presentation by B cells and that at least antigen-uptake involves the activation of tyrosine kinases.

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Abbreviations: Ig, immunoglobulin; PLC, phospholipase C; FITC, fluorescein isothiocyanate; MHC, major histocompatibility complex.

2. MATERIALS AND METHODS

2.1. *Cells and antibodies*

BAL17 B lymphoma cells were maintained in culture medium containing RPMI-1640 supplemented with 10% fetal calf serum, 2 mM glutamine, 100 μg/ml kanamycin, and 50 μM 2-mercaptoethanol. Pretreatment of BAL17 cells with herbimycin A (a gift from Yoshimasa Uehara, National Institute of Health, Japan) was carried out as previously described [6]. A goat IgG-specific T cell clone, 52-3D, was established from BALB/c mice immunized with goat IgG as previously described [15]. Normal goat IgG was purified from normal goat sera by a combination of 50% saturated ammonium sulfate precipitation and a DE 52 column (Whatman Ltd). Goat anti-mouse IgM H chain-specific antibody (anti-IgM) was prepared as described [6]. Goat anti-mouse IgD (anti-IgD) was a gift from Fred Finkelman (Uniformed Services University of Health Sciences, Bethesda, MD). Genistein was from Calbiochem Biochemicals (San Diego, CA).

2.2. *Inositol phosphate production*

Accumulation of inositol phosphate was measured by the method described previously [16].

2.3. *Capping and internalization*

Cells (3×10^5) were attached to a glass slide using Auto-Smear (Hitachi Co.) and stimulated with 5 μg/ml of FITC-labeled anti-IgM (FITC-anti-IgM) on ice. After washing, cells were set in an interactive laser cytometer (ACAS 570, Meridian Instrument Inc.) and fluores-

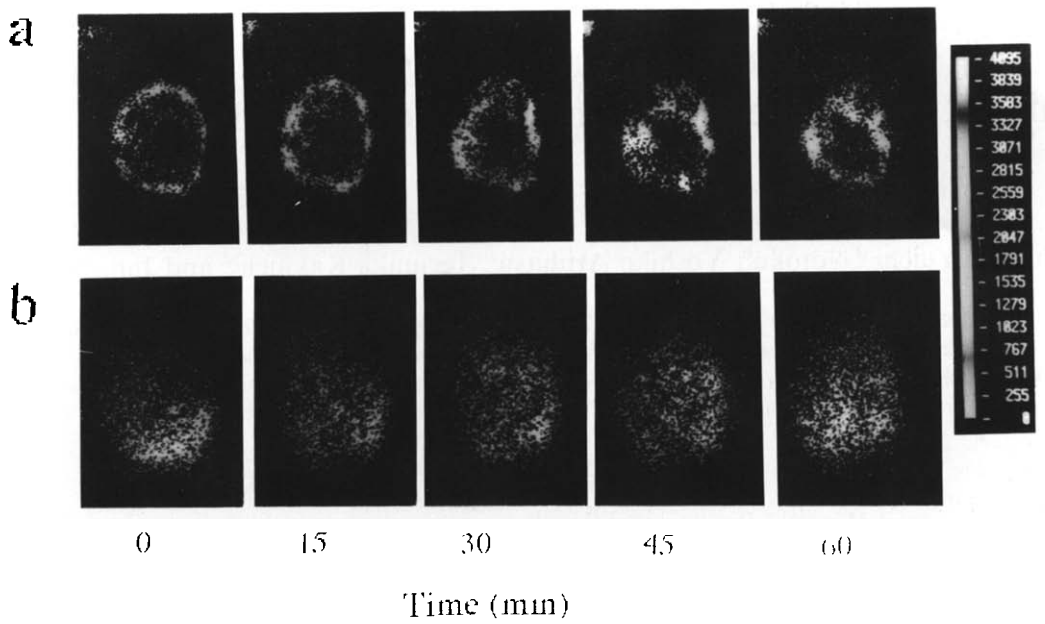

Fig. 1. Herbimycin A inhibits anti-IgM-induced capping of mIg in a single BAL17 cell. BAL17 cells pretreated without (a) or with (b) 1 μM of herbimycin A were stimulated with FITC-anti-IgM on cover slips. A representative single cell was monitored for 60 min

cence was traced every 15 min at 37°C for 60 min. The internalization of FITC-anti-IgM was observed using an ACAS 570 confocal system with a pin-hole of 40 μm. This confocal imaging significantly reduces out-of-focus light using a diffraction limited optics and placing a pin hole in front of the detector to allow only light from the plane of focus to be detected.

Table I

Herbimycin A or genistein inhibits the capacity of BAL17 B lymphoma cells to present anti-IgM or anti-IgD to a goat IgG-specific T cell clone

Antigen	Pretreatment	Accumulation of inositol phosphates (cpm/culture)
Expt 1		
None	−	72
	+	89
Anti-IgM	−	1,084
	+	81
Anti-IgD	−	720
	+	76
Expt 2		
None	−	88
	+	143
Anti-IgM	−	3,480
	+	409

BAL17 B lymphoma cells were pretreated with 1 μM herbimycin A for 12 h (Expt. 1) or 60 μg/ml genistein for 3 h (Expt 2) with the addition of 1 μg/ml of anti-IgM or anti-IgD antibody for the final 3 h. The cells were washed twice with RPMI-1640 and incubated with the [^{3}H]inositol-labeled T cell clone for 40 min The reaction was stopped by the addition of chloroform/methanol Inositol phosphate production was measured as described in section 2

3. RESULTS AND DISCUSSION

We evaluated the role of tyrosine kinase on the capacity of B cells to present an antigen to T cells using the tyrosine kinase inhibitors herbimycin A and genistein [17,18]. Anti-IgM or anti-IgD was used to stimulate B cells since these antigens were efficiently taken up

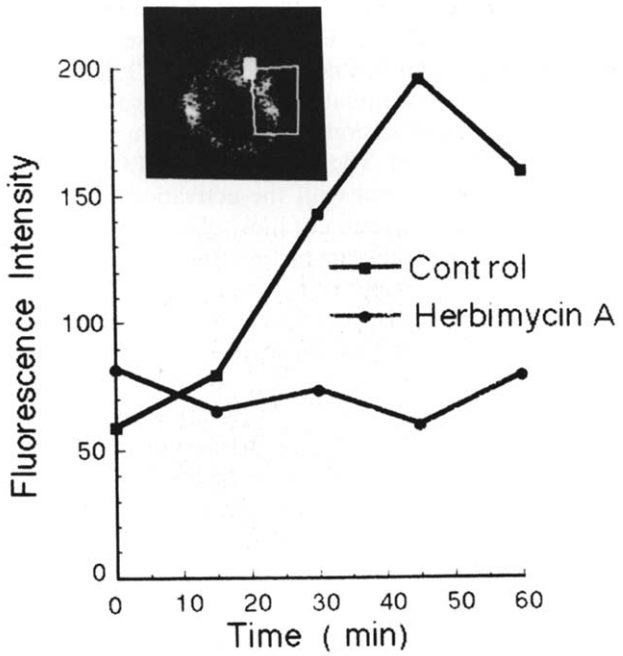

Fig. 2. Fluorescence intensity of a single BAL17 cell pretreated with or without herbimycin A after FITC-anti-IgM stimulation. The fluorescence intensity of a BAL17 cell treated as in Fig. 1 was monitored for 60 min.

through mIg by BAL17 B lymphoma cells bearing mIg and class II molecule. BAL17 cells pulsed with 1 μg/ml anti-IgM or anti-IgD antibody for 3 h caused an increase in inositol phosphate production in a goat IgG-specific T cell clone (Table I). As a control it was shown that goat IgG at 1 μg/ml could not be presented to T cells (data not shown). When BAL17 cells were pretreated with 1 μM herbimycin A for 12 h or 60 μg/ml genistein for 3 h, the ability of the BAL17 cells to present the antigen was markedly blocked (Table I).

B cells take up an antigen and process it. The peptide generated binds to major histocompatibility complex (MHC) class II molecules where it is recognized by T cells in the context of the MHC molecule [1,2]. Tyrosine kinase inhibitors are thought to inhibit at least one of these events. As a first step in analyzing the role of tyrosine kinase on these events, we examined whether inhibitors affect the anti-Ig-mediated capping or internalization of mIg. BAL17 cells were incubated with FITC-anti-IgM at 37°C for 60 min using ACAS 570, which allows monitoring the fate of FITC-anti-IgM at the single cell level. Significant capping was observed at 15 min and became more prominent at 45 min after FITC-anti-IgM stimulation (Fig. 1). Pretreatment with herbimycin A (1 μM) or genistein (30 μg/ml) markedly inhibited the capping. The extent of capping was quantitated by measuring the relative fluorescence intensity around the capped area. Complete inhibition of capping is observed over the 60 min so far examined (Fig. 2). The dose–effect of the inhibitor was examined. As shown in Fig. 3, herbimycin A at 0.3 μM significantly inhibited the capping with approximately 90% inhibition observed at 1 μM; complete inhibition (more than 95%) was achieved at 3 μM. The profile of this dose–effect is quite similar to that of the sensitivity to anti-Ig-

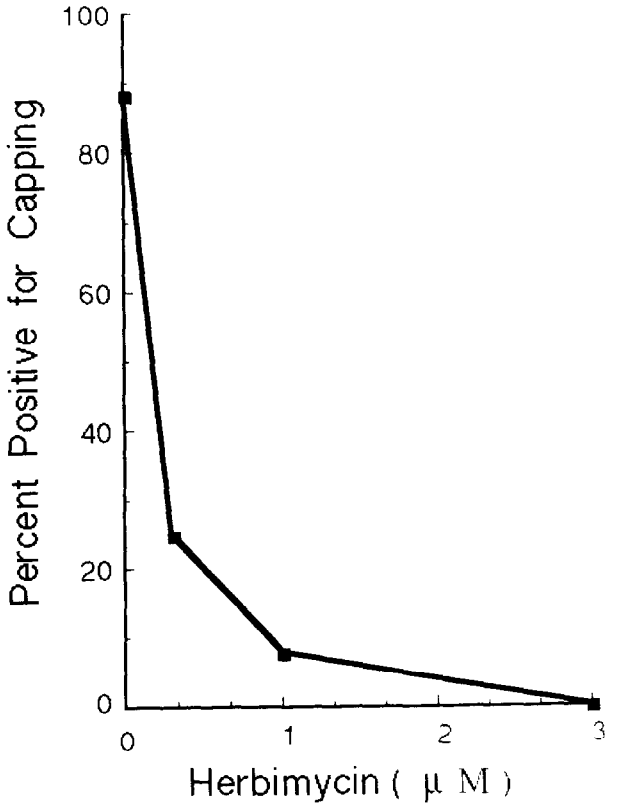

Fig. 3. Dose–response of herbimycin A-mediated inhibition on anti-IgM-induced capping. BAL17 cells pretreated with or without various concentrations of herbimycin A for 12 h were stimulated with FITC-anti-IgM for 50 min. Percentage of cells forming caps was calculated as follows: (cell number forming caps/total cell number) × 100; more than 100 BAL17 cells were examined. Identical results were obtained in two other experiments.

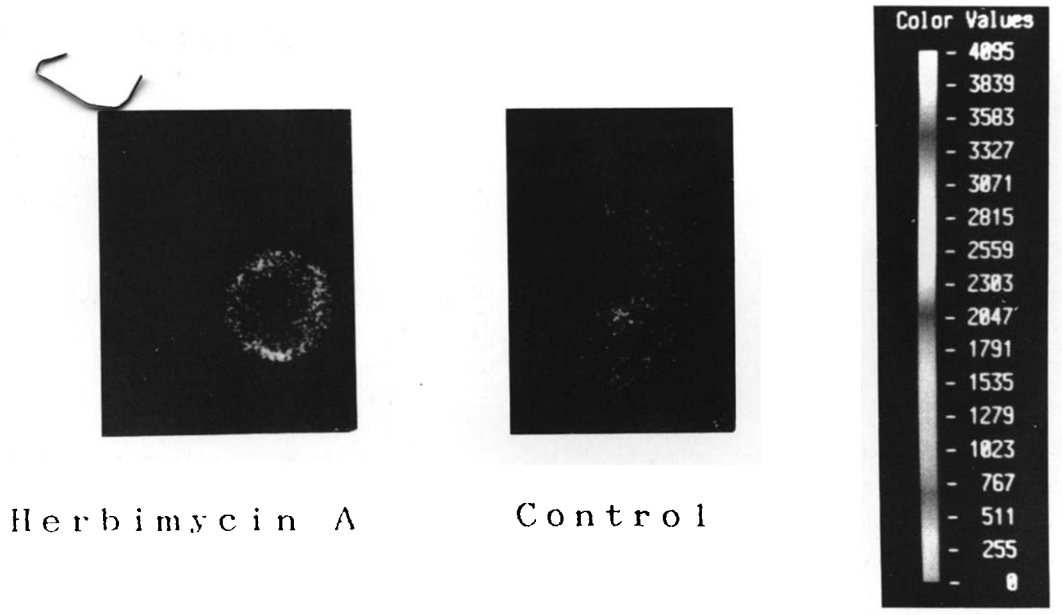

Fig. 4. Herbimycin A inhibition of anti-IgM-induced endocytosis.

induced inositol phospholipid metabolism, as reported previously [6]. This kinase inhibitor-mediated suppression of capping was also observed using ^{125}I-labeled goat anti-IgM antibody (data not shown). To check the anti-IgM-mediated internalization accurately, we used ACAS 570 under confocal conditions. As shown in Fig. 4, significant fluorescence from the inside of FITC-anti-IgM-treated BAL17 cells was observed, whereas no fluorescence was observed inside the herbimycin A-pretreated cells. This indicates that tyrosine kinase participates in anti-Ig-mediated internalization.

The present results indicate that tyrosine kinase is involved in the capacity of B lymphoma cells to present antigen to T cells, partly because of the participation of the kinase in mIg-mediated internalization events. These findings support the recent report using conventional microscopy that the kinase inhibitor tyrphostin inhibits anti-Ig-mediated endocytosis in normal resting B cells [19]. We and others have demonstrated that several proteins are phosphorylated after receptor cross-linkage of mIg in BAL17 cells [6,12]. The dose response profile for the herbimycin A-mediated inhibition of anti-IgM-induced tyrosine phosphorylation is similar to that for anti-IgM-induced capping. B cells have been shown to display at least three src-related tyrosine kinases [9–11], one of which might participate in these processes. Interestingly, the p53^{lyn} protein is down-regulated after anti-Ig stimulation while p56^{lyn} content remains unchanged in B lymphoma cells [12]. Down-regulation of the kinase might be accompanied by down-modulation of mIg through a phosphorylation event. ACAS may provide a valuable tool with which to monitor the regulation by tyrosine kinase of the capping or internalization of mIg at the single cell level.

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