Volume 284, number 2, 249–251 FEBS 09849 June 1991

ADONIS 001457939100559N

Antigen receptor-mediated calcium signals in B cells as revealed by confocal fluorescence microscopy

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Received 26 March 1991

A confocal fluorescence microscope was used to study the antigen receptor-mediated calcium signals in B cells. Anti-IgD binding to B lymphoma cells (BAL17) increased the intracellular calcium concentration with short lag times. Confocal fluorescence images of the fluo-3-loaded BAL17 cells showed that the intracellular calcium ion concentrations increased non-homogeneously, suggesting that the calcium signals transferred not only to the cytoplasm but also to the nucleus.

Confocal fluorescence microscope; Calcium signal; Nucleus; B cell; Antigen receptor

1. INTRODUCTION

In virtually all systems in which ligand binding to receptors induces increased polyphosphoinositide hydrolysis, ligand binding also induces a rapid increase in intracellular free calcium levels [1–3]. In these systems, the intracellular free calcium concentration is usually measured by fluorescent Ca^{2+} indicators [3]. In addition, recently, a digital fluorescence microscope together with the use of fluorescent Ca^{2+} indicators has enabled us to measure the intracellular free calcium concentration in an individual cell [4–8]. However, a conventional fluorescence microscope is not suited to study the heterogeniety of the intracellular calcium distribution because of its poor resolution [9,10]. Compared to conventional light microscopy, confocal scanning microscopy has a more superior capability for directly visualizing the three-dimensional structure of biological objects [9–12]. Thus, we have observed a single cell to study the calcium signals in B cells using a confocal fluorescence microscopy. To analyze the localization of calcium signals induced by receptor cross-linkage, confocal fluorescence images were used.

B lymphoma cells (BAL17 cells) possess the membrane forms of both immunoglobulin M (IgM) and immunoglobulin D (IgD) [13,14]. Cross-linkage of the membrane immunoglobulin in the form of cell suspensions has been shown to induce an increase in inositol phospholipid metabolism and intracellular free calcium concentration. In the present experiment, we observed the calcium signals in an individual BAL17 cell using a confocal fluorescence microscope. Confocal fluorescence images suggested that anti-IgD binding to BAL17 cells transferred signals not only to the cytoplasm but also to the nucleus.

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2. MATERIALS AND METHODS

B lymphoma cells (BAL17 cells) were maintained in a culture medium containing RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% FCS (fetal calf serum), streptomycin (100 μg/ml), penicillin G (100 U/ml), L-glutamine (2 mM), and 2-mercaptoethanol (50 μM).

Single cell observation by confocal fluorescence microscopy was done according to the following procedure. BAL17 cells were harvested from culture dishes and they were transferred to an observation chamber which was coated with 2.5 μg/ml of poly-L-lysine (Sigma, St. Louis, MO). The chamber was then placed in a CO_2 incubator for 3 h. After incubation, cells were treated with a medium containing 5 μM of fluo-3-AM (Dojindo, Kumamoto Japan) for 40 min. They were then washed with HEPES buffer (10 mM HEPES, 140 mM NaCl, 5 mM KCl and 1 mM $CaCl_2$ at pH 7.2) to remove free fluo-3-AM in solution.

Confocal fluorescence microscopic images of BAL17 cells were taken under a confocal scanning fluorescence microscope system (MRC-600, BioRad) with an inverted epifluorescence microscope (Nikon TMD-EFQ). The temperature of the observation chamber was controlled at 37°C. A conventional digital imaging fluorescence microscope was used in combination with an inverted epifluorescence microscope (Nikon TMD-EFQ) with a silicon intensified target TV camera (Hamamatsu photonics 2400-08) and a U-matic video tape recorder (Sony). The fluorescence images were analyzed with a digital image processor (Image Sigma II and TVIP-2000, Avionics), which was controlled by a microcomputer (NEC PC-9801VX) [5,7].

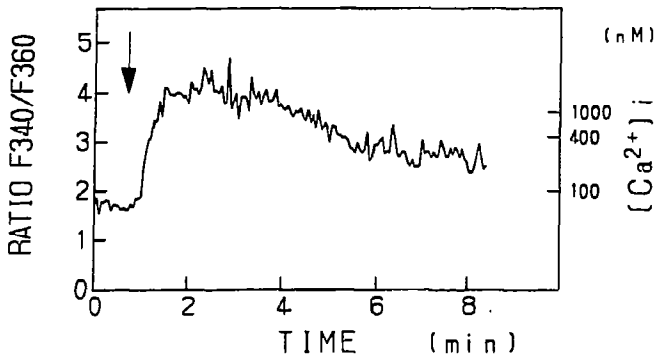

Fig. 1. Time course of the change of the $[Ca^{2+}]_i$ in a fura-2-loaded BAL17 cell in response to anti-IgD (8 μg/ml) at 37°C. Ratios of the fluorescence images at 340 and 360 nm were taken at intervals of 1.6 s. The arrow indictes the time at which anti-IgD was added.

3. RESULTS AND DISCUSSION

First, we measured the time courses of the intracellular free calcium concentration $[Ca^{2+}]_i$ in an individual BAL17 cell using a conventional digital imaging fluorescence microscope. One of the results is shown in Fig. 1. Fura-2-loaded BAL17 cells were stimulated with anti-IgD (Hδa/1, 8 μg/ml), after which we observed the fluorescence images. Ratios of fluorescence images at 340 nm and 360 nm gave $[Ca^{2+}]_i$ images. The $[Ca^{2+}]_i$ images of an individual cell seemed to be homogeneous as if the concentration of calcium ions were of a similar level everywhere in the cell. We measured the time courses of the $[Ca^{2+}]_i$ images. The abrupt elevation of the $[Ca^{2+}]_i$ in BAL17 cells was observed as shown in Fig. 1.

Next, we observed the confocal fluorescence images of fluo-3-loaded BAL17 cells as shown in Fig. 2. The fluorescence images (excitation at 488 nm) of BAL17 cells before stimulation gave weak and non-homogeneous ones (Fig. 2A). After anti-IgD stimulation, the fluorescence intensity of the fluo-3-loaded BAL17 cells abruptly increased with short lag times (Fig. 2B). Fig. 2B shows that the fluorescence intensity of the fluo-3-loaded BAL17 cells became much greater and more non-homogeneous than that before the stimulation (Fig. 2A). From the morphological pattern, the parts of the bright fluorescence seemed to belong to the nucleus in BAL17 cells. These results suggest that the anti-IgD binding to BAL17 cells transferred calcium signals not only to the cytoplasm but also to the nucleus.

At the present time, we have ascertained that the fluorescence intensity observed by confocal fluorescence microscopy is solely proportional to the concentration of calcium ions. However, the changes in fluorescence after stimulation were abrupt and quite similar to the pattern of the time courses of the $[Ca^{2+}]_i$ observed by conventional fluorescence microscopy. These results indicate that the fluorescence intensity of the fluo-3-loaded BAL17 cells observed by confocal fluorescence microscopy reflected mostly the concentration of calcium ions inside the cells. Thus, the present results suggest that the antigen receptor-mediated calcium signals in B cells transferred not only to the cytoplasm but also to the nucleus.

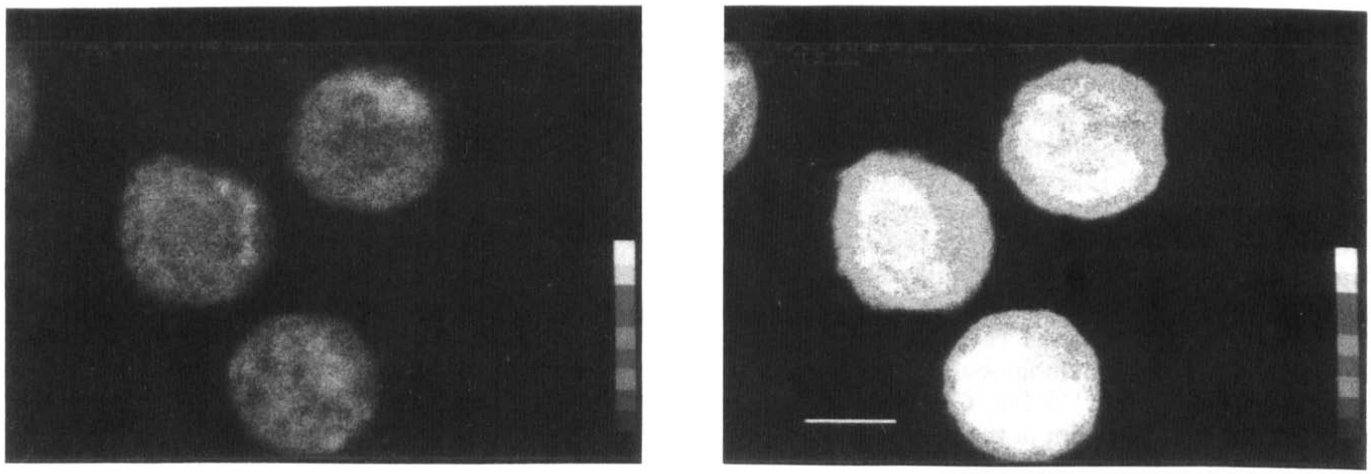

Fig. 2. Confocal fluorescence images of fluo-3-loaded BAL17 cells before and after anti-IgD stimulation at 37°C. The fluorescence images were observed at the excitation wavelength of 488 nm. A. Confocal fluorescence images of BAL 17 cells before stimulation. B. Confocal fluorescence images of BAL 17 cells after anti-IgD (8 μg/ml) stimulation. The picture was observed 1 min after anti-IgD stimulation.

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