

## Difference in the $\text{Ca}^{2+}$ Responses of Various Human T Cell Lines to Stimulation of the T Cell Receptor/CD3 Complex with Monoclonal Antibodies<sup>1</sup>

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### SUMMARY

The surface expression of the CD3 complex and the change of the intracellular free  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) following stimulation with monoclonal antibodies against T cell receptor (TcR)/CD3 complex are determined in several human T cell leukemia lines, Jurkat, TALL-1 (sublines 1 and 2), HPB-ALL, HPB-MLT, CCRF-CEM, and PEER. All the lines showed the  $[\text{Ca}^{2+}]_i$  response to an anti-CD3 monoclonal antibody, OKT3, but the magnitude of the response was notably different among the lines used: Jurkat and TALL-1 (subline 2) had markedly large responses, TALL-1 (subline 1) and PEER had relatively large responses, while CCRF-CEM, HPB-ALL and HPB-MLT had relatively small responses. No correlation was found between the surface expression of the TcR/CD3 complex and the  $[\text{Ca}^{2+}]_i$  response. The  $[\text{Ca}^{2+}]_i$  response found in TALL-1 (subline 1) had only the early spike component, which was independent of extracellular  $\text{Ca}^{2+}$ , and no influx phase of the  $[\text{Ca}^{2+}]_i$  response, which was dependent on the extracellular  $\text{Ca}^{2+}$ .

**Key words:** Human T cell leukemia lines, T cell receptor/CD3 complex, Intracellular free  $\text{Ca}^{2+}$  concentration, Monoclonal antibodies

### INTRODUCTION

The immunological specificity of T lymphocytes is determined by the binding of the T cell receptor (TcR)/CD3 complex to the complex between major his-

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tocompatibility complex (MHC) class I or class II molecule and antigen fragment on target or antigen presenting cells. These recognition/binding and subsequent secondary interactions between T lymphocytes and target or antigen presenting cells activates T lymphocytes. The transmembrane signals generated as a result of the stimulation of the TcR/CD3 complex is at least partly reproduced by the binding of an antibody to the TcR/CD3 complex. Thus, it has been shown that monoclonal antibodies against TcR and CD3 complex induce the hydrolysis of phosphatidylinositol 4,5-bisphosphate and an increase in the intracellular free  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) in T lymphocytes. A human T cell leukemia line, Jurkat, has been used most commonly as a model system in the analysis of the signal transduction through the TcR/CD3 complex. In the present paper, we compared various human T cell lines, which express the TcR/CD3 complex on the cell surface, for the  $[Ca^{2+}]_i$  response to anti-TcR and anti-CD3 monoclonal antibodies.

## MATERIALS AND METHODS

### *Antibodies*

OKT3 (mouse IgG<sub>2a</sub>) and KT38 (mouse IgG<sub>1</sub>) monoclonal antibodies were prepared from ascites of the hybridoma-bearing mice by ammonium sulfate precipitation and chromatography on protein A-Sepharose CL-4B (Sigma). The OKT3 hybridoma was obtained from the American Type Culture Collection. The KT38 hybridoma was previously described(12). Anti Leu-4 (mouse IgG<sub>1</sub>) monoclonal antibody was obtained from Becton Dickinson Monoclonal Center, Inc. (Mountain View, Calif., U. S. A.). WT31 (mouse IgG<sub>1</sub>) monoclonal antibody was purchased from Sanbio (Amuden, Netherlands). A purified immunoglobulin fraction (10 mg protein/ml) of rabbit anti-mouse immunoglobulins was obtained from Dakopatts (Glostrup, Denmark).

### *Cell Culture*

The T cell leukemia lines, Jurkat (clone E6-1), TALL-1 (subline 1), TALL-1 (subline 2), HPB-ALL, HPB-MLT, CCRF-CEM, and PEER were cultured in Iscove's modified Dulbecco's medium (Sigma) supplemented with 10% heat-inactivated (56°C for 30 min) fetal calf serum, 2 mM glutamine, 1 mM sodium pyruvate, 50 U/ml penicillin, and 50  $\mu$ g/ml streptomycin.

### *Determination of $[Ca^{2+}]_i$*

Cells were harvested at late log phase of the growth by centrifugation. The cells were loaded with fura 2 by incubating the cells ( $1.5 \times 10^7$ /ml in serum-free medium) with 3  $\mu$ M fura 2/acetoxymethyl ester at 37°C for 30 min. After load-

ing, the cells were washed once with serum-free medium and twice with HEPES-buffered saline containing 1.0 mM Ca<sup>2+</sup>. The cells were finally suspended in the same saline at  $2 \times 10^6$ /ml. Changes in fura 2 fluorescence were recorded at 37°C as described (3, 8, 11). Excitation and emission wavelengths were 340 and 500 nm with 2- and 10-nm slits, respectively. Monoclonal antibodies in a volume of 1–10  $\mu$ l were added as stimuli to 1 ml of fura 2-loaded cells. In some experiments, extracellular Ca<sup>2+</sup> was depleted by suspending fura 2-loaded cells in Ca<sup>2+</sup>, Mg<sup>2+</sup>-free HEPES-buffered saline containing 1 mM EGTA just before the [Ca<sup>2+</sup>]<sub>i</sub> determination. [Ca<sup>2+</sup>]<sub>i</sub> was calculated by the ratio of the fura 2 fluorescence intensities at two excitation wavelengths (340 and 360 nm) as described by Grynkiewicz *et al.*(2).

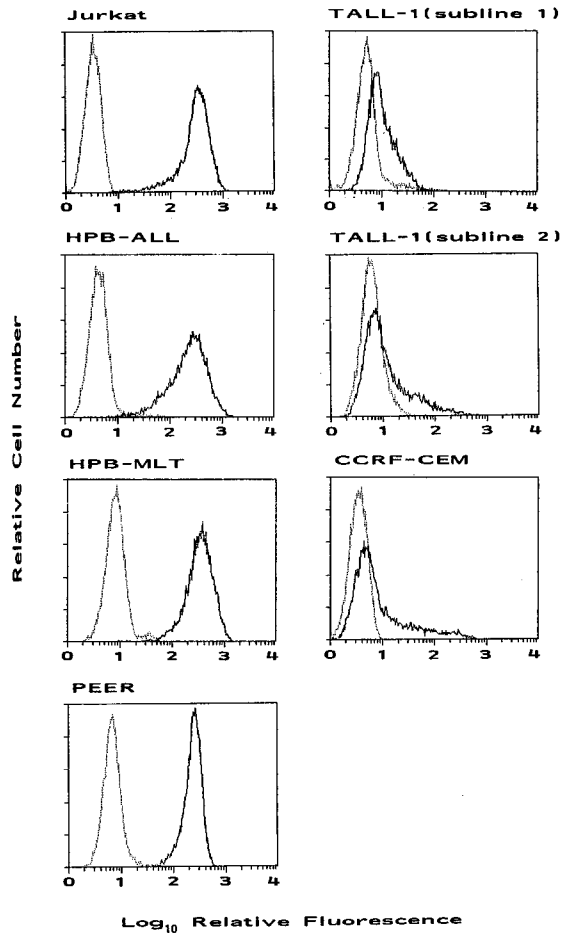
#### Flow cytometric analysis

Cells ( $5 \times 10^5$ – $1 \times 10^6$ ) were incubated at 4°C for 30 min with either OKT3 or KT38 (1–2  $\mu$ g/100  $\mu$ l) in 100  $\mu$ l of phosphate-buffered saline containing 0.1% bovine serum albumin and 0.1% sodium azide. After washing twice in 2 ml of incubation medium, cells were resuspended in 100  $\mu$ l of the same medium containing fluorescein-coupled goat anti-mouse immunoglobulin F(ab')<sub>2</sub> (Tago, Burlingame, U.S.A.) and further incubated at 4°C for 30 min. Following three washings, cells were suspended in 1 ml of incubation medium and 10,000 cells/sample analyzed in flow cytometry using a FACScan cell analyzer. The background fluorescence was determined by omitting the first antibody in the staining procedure.

## RESULTS AND DISCUSSION

The surface expression of the CD3 complex was determined by the binding of an anti-CD3 monoclonal antibody, OKT3, to each cell line (Fig. 1). Among the cell lines used, HPB-ALL, HPB-MLT, Jurkat, and Peer express a relatively large amount of the CD3 complex, whereas CCRF-CEM and TALL-1 (sublines 1 and 2) express relatively small amount of the CD3 complex. The CD3 complex and TcR are known to be coexpressed on the cell surface(1). The monoclonal antibody KT38 is an antibody with clonotype specificity(12). In Jurkat and TALL-1 (subline 2), the TcR expression determined by the KT38 binding correlates well with the CD3 expression determined by the OKT3 binding (data not shown).

The change of [Ca<sup>2+</sup>]<sub>i</sub> following stimulation with monoclonal antibodies against CD3  $\epsilon$  chain(13), OKT3, anti Leu-4, and WT31, and against TcR, KT38, was measured in various human T cell lines by the use of fluorescent Ca<sup>2+</sup> indicator Fura 2. The magnitude and time course of [Ca<sup>2+</sup>]<sub>i</sub> increase following the



**Fig. 1** Surface expression of CD3 on various human T cell lines. The indicated cell lines were incubated with OKT3. Bound antibody was detected with fluorescein-coupled second antibody (thick line). Nonspecific fluorescence was assessed by omitting monoclonal antibody for the first incubation step (thin line). Histograms represent the number of cells (ordinate) vs.  $\log_{10}$  relative fluorescence intensity.

antibody binding are different among the cell lines used. The same cell line responds in different manners to OKT3 and WT31. OKT3 always induces larger  $[Ca^{2+}]_i$  response than WT31. The response to KT38 found in Jurkat and TALL-1 (subline 2) was similar to the response to OKT3 but not identical with the response. The  $[Ca^{2+}]_i$  response found by stimulation with anti Leu-4 was also similar to the response to OKT3 but not identical with the response.

Among the cell lines examined, Jurkat and TALL-1 (subline 2) have mark-

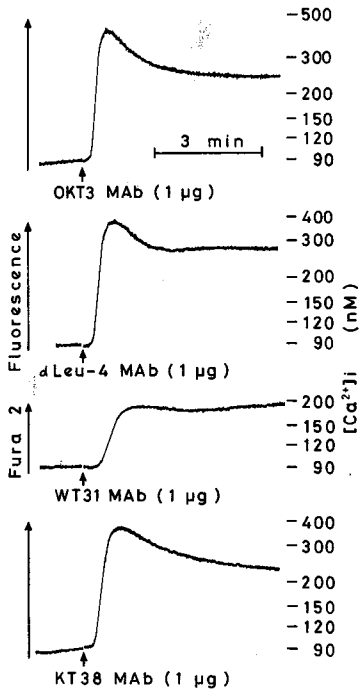


Fig. 2 Effects of OKT3, anti Leu-4, WT31, and KT38 on [Ca<sup>2+</sup>]<sub>i</sub> of Jurkat cells in the presence of extracellular Ca<sup>2+</sup>.

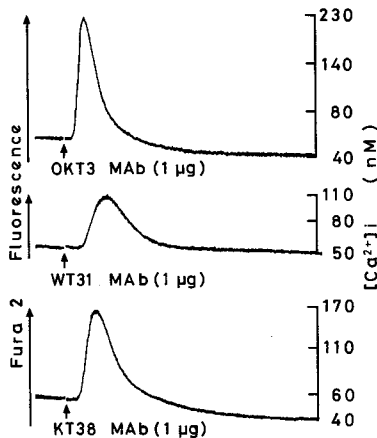


Fig. 3 Effects of OKT3, WT31, and KT38 on [Ca<sup>2+</sup>]<sub>i</sub> of Jurkat cells in the absence of extracellular Ca<sup>2+</sup>.

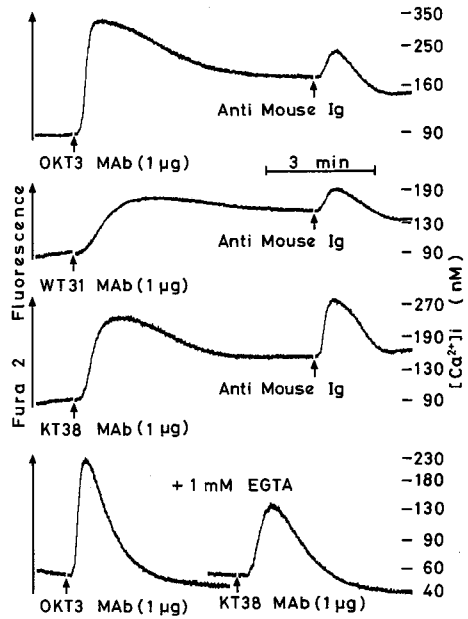


Fig. 4 Effects of OKT3, WT31, KT38 and anti-mouse immunoglobulin on [Ca<sup>2+</sup>]<sub>i</sub> of TALL-1 (subline 2) cells in the presence or absence (indicated by +1mM EGTA) of extracellular Ca<sup>2+</sup>.

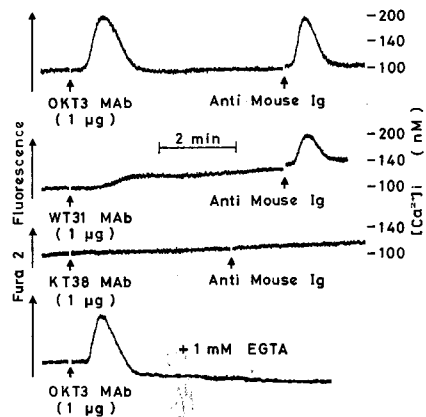
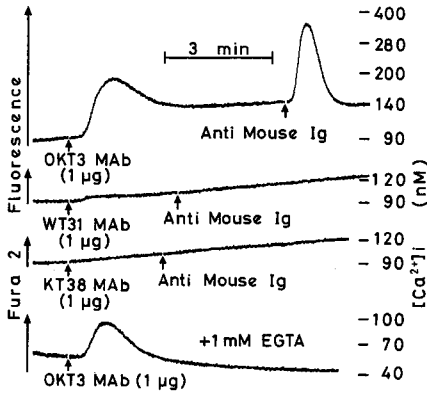
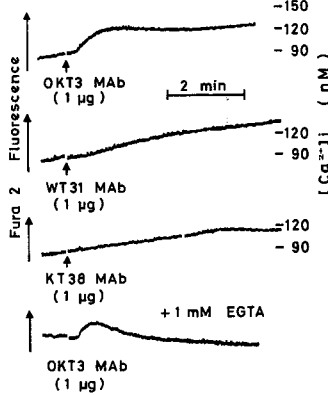


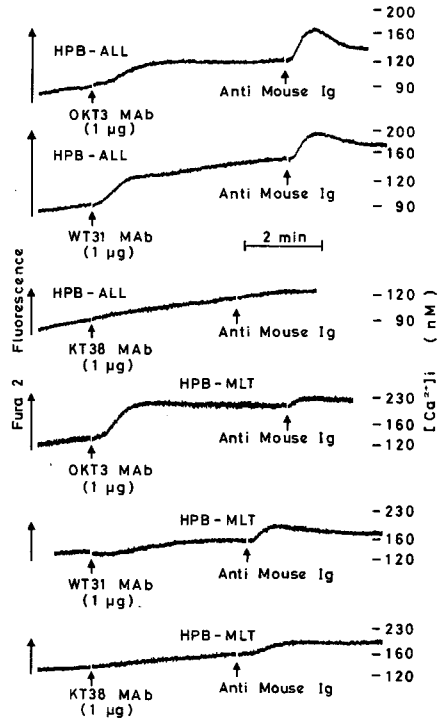
Fig. 5 Effects of OKT3, WT31, KT38 and anti-mouse immunoglobulin on [Ca<sup>2+</sup>]<sub>i</sub> of TALL-1 (subline 1) cells in the presence or absence (indicated by +1mM EGTA) of extracellular Ca<sup>2+</sup>.



**Fig. 6** Effects of OKT3, WT31, KT38 and anti-mouse immunoglobulin on  $[Ca^{2+}]_i$  of PEER cells in the presence or absence (indicated by +1mM EGTA) of extracellular  $Ca^{2+}$ .



**Fig. 7** Effects of OKT3, WT31 and KT38 on  $[Ca^{2+}]_i$  of CCRF-CEM cells in the presence or absence (indicated by +1mM EGTA) of extracellular  $Ca^{2+}$ .



**Fig. 8** Effects of OKT3, WT31, KT38 and anti-mouse immunoglobulin on  $[Ca^{2+}]_i$  of HPB-ALL and HPB-MLT cells in the presence of extracellular  $Ca^{2+}$ .

edly large  $[Ca^{2+}]_i$  response to OKT3, WT31, and KT38 (Figs. 2 and 4). The response seems to consist of two components; an early spike independent of extracellular  $Ca^{2+}$  and a later sustained level of increased  $[Ca^{2+}]_i$  dependent on extracellular  $Ca^{2+}$  (Fig. 2 and 3). TALL-1 (subline 1) and PEER have relatively large  $[Ca^{2+}]_i$  responses to OKT3 (Figs. 5 and 6). TALL-1 (subline 1) also re-

sponded to WT31 but not to KT38 (Fig. 5). The lack of response of TALL-1 (subline 1) to KT38 is a result difficult to explain if we assume both sublines 1 and 2 of TALL-1 originate from the same clone and KT38 is a monoclonal antibody with clonotype specificity. The molecule recognized by the monoclonal antibody KT38 and the clonal relationship between sublines 1 and 2 of TALL-1 are now the subjects under investigation in our laboratory. The  $[Ca^{2+}]_i$  response found in TALL-1 (subline 1) has only the spike component and no influx phase of the  $[Ca^{2+}]_i$  response (Fig. 5). PEER has a TcR with  $\gamma$  and  $\delta$  chains (6, 14), with which the monoclonal antibody WT31 has a poor reactivity. CCRF-CEM, HPB-ALL, and HPB-MLT have relatively small  $[Ca^{2+}]_i$  responses to OKT3 and WT31 (Figs. 7 and 8).

These results indicate that the  $[Ca^{2+}]_i$  response, and by inference the activation of phospholipase C, found by stimulation of T cell lines with monoclonal antibodies against the TcR/CD3 complex do not have a direct correlation with the amount of the TcR/CD3 complex expressed on the cell surface. Further investigation is required to identify the factors involved in the coupling of the TcR/CD3 complex with the signal transducing components.

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