Galectin-3 stimulates uptake of extracellular Ca^{2+} in human Jurkat T-cells

Sucai Dong, R. Colin Hughes*

National Institute for Medical Research, Mill Hill, London NW7 1AA, UK

Received 15 July 1996

Abstract Galectin-3, a mammalian galactoside-binding protein, is not expressed in the Jurkat T-lymphoblastoid cell line. However, Jurkat cells express surface glycoprotein receptors for galectin-3, one of which is shown to be the glycosylated heavy chain of CD98 (4F2 antigen), a T-cell activation marker. Addition of galectin-3 to Jurkat cells triggers a sustained influx of extracellular Ca^{2+} in a concentration dependent manner. The induced increase in cytosolic $[Ca^{2+}]_i$ is blocked by sugar hapten inhibitors of galectin-3. The galectin-3-induced effect is insensitive to voltage-gated Ca^{2+} channel antagonists such as prenylamine, nifedipine and diltiazem and to pertussis toxin but is inhibited by cholera toxin. The results suggest that galectin-3 released by accessory cells such as macrophages may bind in vivo to T-cell activation antigens and also participate in Ca^{2+} signalling.

Key words: Jurkat T-cell; Galectin-3; Extracellular Ca^{2+}

1. Introduction

Galectin-3, also called Mac-2 surface differentiation antigen [1], is a member of a family of mammalian carbohydrate-binding proteins and is expressed widely in epithelial cells and some leukocytes [2,3]. Galectin-3, like other galectin family members, lacks a signal sequence for import into the endoplasmic reticulum and is secreted from the cytoplasm by a novel mechanism independent of the classical secretary pathway [4,5]. Secreted galectin-3 binds to appropriately glycosylated components of the extracellular matrix such as laminin and fibronectin isoforms and to selected cell surface glycoproteins [5,6].

Galectin-3 is reported to be a signalling molecule. It binds to IgE as well as the high affinity IgE receptor on rat basophilic leukemia cells and triggers degranulation and serotonin release [7,8]. In human neutrophils, galectin-3 may play a role in IgE-mediated activation and stimulates superoxide production [9]. Galectin-3 also potentiates IL-1 production by human monocytes [10]. These results together suggest that galectin-3 may modulate various inflammatory responses associated with monocytes/macrophages, mast cells, neutrophils and eosinophils. In this context galectin 3 secretion from different cell types including macrophages is increased markedly by heat shock as well as by Ca^{2+} ionophores [4,5].

Recently we have identified major plasma membrane glycoproteins recognized by galectin-3 on the mouse WEHI-3 macrophage cell line and on thioglycollate-elicited peritoneal macrophages [11]. These receptors include the α-subunit of CD11b/CD18 integrin, also known as Mac-1 antigen [12], and the heavy chain of CD98 recognized by the 4F2 and RL388 monoclonal antibodies in human [13,14] and rodent [15,16] cells, respectively. CD98 antigen is expressed on most cell lines in culture as well as on monocytic cells. Interestingly, CD98 is also expressed on activated human lymphocytes although not on resting T and B lymphocytes. Although preliminary evidence [17] suggests that lymphocytes do not express galectin-3, we were prompted by these findings to examine whether lymphocytes bind exogenous lectin. In this report, we show that exogenous galectin-3 binds to surface glycoproteins, including CD98, of the human lymphoblastoid Jurkat T-cell line. Furthermore, surface ligation of these cells triggers an increase in intracellular Ca^{2+}, suggesting that activated lymphocytes may be responsive to galectin 3 released from other cell types.

2. Materials and methods

2.1. Antibodies and lectins

Rat monoclonal antibody M3/38 against mouse galectin-3 (Mac-2 antigen) was from Boehringer Mannheim, East Sussex, UK. Polyclonal rabbit antisera against hamster galectin-3 N-terminal and C-terminal domains were obtained as described [18], and shown by Western blotting to cross-react with human galectin-3. Mouse monoclonal antibodies against human CD98 (clone 4F2) and CD11b/CD18 (clone 44) subunits were from PharMingen, Cambridge, UK. Second antibodies and conjugates were from Sigma. Recombinant hamster galectin-3 and the carbohydrate recognition domain CRD comprising the C-terminal half of the lectin were obtained as described [18]. *Tetracarpidium conophorum* agglutinin TCA and its biotinylated derivative were as described [19]. *Griffonia simplicifolia* isolectin GSL-IB4, its FITC derivative and FITC-labelled streptavidin were from Sigma.

2.2. Cell culture and labelling

Human Jurkat T cells were grown in suspension cultures at 37°C in RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum. For metabolic labelling, cells were washed twice with methionine-free medium and subsequently cultured for up to 16 h in the same medium supplemented with 200 μCi/ml Trans ^{35}S label (Amersham International, UK). Cells were harvested by centrifugation at $3000 \times g$ for 15 min at 2°C. Human monocytic leukemia THP-1 cells were cultured in RPMI 1640 medium with 15% fetal calf serum and 50 nM TPA (12-*O*-tetradecanoylphorbol-1,3-acetate) at 37°C for 3 days.

2.3. Affinity chromatography

Recombinant hamster galectin-3 was coupled to CNBr-activated Sepharose 4B (Pharmacia LKB) in 150 mM lactose-containing buffer and the product containing approx. 1 mg of lectin per ml of beads was packed into a 10 ml column and washed extensively with 8 mM CHAPS in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 100 μM leupeptin, 1 μM pepstatin, 10 μg/ml aprotinin, 1 mM PMSF (buffer A). Labelled cells (approx. 10^7) were lysed in 40 mM CHAPS in buffer A at 2°C and the supernatant (2 ml) after centrifugation at $100\,000 \times g$ for 1 h was applied to the galectin-3 column. Fractions (2 ml) were eluted first with 8 mM CHAPS-buffer A (160 ml) followed by 150 mM lactose-8 mM CHAPS-buffer A (20 ml). Fractions were analyzed for radioactivity and peak fractions were pooled and concentrated to 2 ml.

*Corresponding author. Fax: (44) (0181) 913 8595.

PII S0014-5793(96)01031-9

2.4. Immunoprecipitation and Western blotting

Samples (0.4 ml) of cell lysates or column fractions diluted 1:3 in 10% Nonidet P40, 0.5% deoxycholate, 0.1% SDS, 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 100 μM leupeptin, 1 μM pepstatin, 10 μg/ml aprotinin, 1 mM PMSF (buffer B) were pre-cleared by addition of normal rabbit serum (80 μl) followed by a 50% (w/w) suspension of Protein A-Sepharose in buffer B (50 μl) and incubation for 2 h at 2°C. Cleared supernatants obtained after centrifugation were then treated sequentially with various monoclonal antibodies. After addition of one primary antibody (1:100 dilution) and incubation overnight at 2°C, polyclonal rabbit anti-rat or -mouse Ig (1:100 dilution) was added and immune complexes were collected on Protein A-Sepharose (50 μl) after 2 h at 2°C. The beads were washed five times with buffer B, once with 50 mM Tris pH 8.0 and immune complexes were dissolved in 100 μl of Laemmli buffer [20] for SDS-7.5% PAGE under reducing or non-reducing conditions. Bands were located by fluorography. In other experiments, Western blotting of cell lysates was performed as described [6].

2.5. Flow cytometry

Cells were washed and suspended in PBS, 0.1% BSA, 0.1% NaN_3(PAB) and incubated with monoclonal antibody at saturating concentrations (10 μg/ml) at 2°C for 30 min. Incubation with FITC-second antibody diluted 1:30 in PAB was for 30 min. The cells were again washed in PAB and analyzed by a FACStar^{Plus} (Becton-Dickinson) after fixation in 1% paraformaldehyde-PBS for 10 min. In other experiments cells were treated with FITC-GSL-IB4 or with biotinylated TCA (each 30 μg/ml in PAB). In the latter case, bound TCA was revealed by incubation with FITC Streptavidin (10 μg/ml in PAB).

2.6. Measurement of $[Ca^{2+}]_i$

Cells (10^6) were loaded in culture medium with the intracellular fluorescence indicator Indo1-AM (1 μM, acetoxymethyl ester, Sigma) at 37°C for 30 min [21]. Cells were washed and re-suspended (5×10^6 cell/ml) in 10 mM HEPES pH 7.4, 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgSO_4$, 5 mM glucose (buffer C). Ca^{2+} mobilization was measured at 37°C for each sample (0.4 ml) as indicated in Section 3 in a Perkin-Elmer Luminescence Spectrometer LS50 using excitation and emission wavelengths of 355 nm and 405/480 nm. Fluorescence intensities for Ca^{2+}-saturated and Ca^{2+}-free dye, respectively, were determined by lysing the cells with 0.1% Triton X-100 in buffer C followed by addition of 10 mM EGTA and 400 mM Tris to the lysed cell suspension. Galectin-3 at various final concentrations was added to cells after 1.5 min of continuous recording when the fluorescence signal had stabilized. In other experiments, Indo 1-AM-loaded cells were washed and resuspended in Ca^{2+}-free buffer C or were treated with various concentrations of diltiazem, nifedipine or prenylamine (Sigma) in Ca^{2+}-containing buffer C for 2 min at 37°C before addition of galectin-3 (40 μg/ml). Other cell aliquots (10^6) were cultured in medium containing pertussis toxin or cholera toxin at 37°C for 16 or 3 h, respectively. The cells were then loaded with Indo 1-AM and treated with galectin-3 (40 μg/ml).

3. Results and discussion

3.1. Jurkat T cells do not express galectin-3

No surface expression of galectin-3 was found in Jurkat cells by flow cytometry using the rat M3/38 monoclonal antibody raised against mouse lectin (Fig. 1a, line 1). Similar findings were obtained using rabbit polyclonal antibodies [18] against hamster galectin-3 (results not shown). Western blotting of cell lysates confirmed that Jurkat cells produce no galectin-3 (Fig. 2). As positive control we used human monocytic cells THP-1 that express low but detectable levels after TPA treatment (Fig. 2).

Table 1
Effects of inhibitors on $[Ca^{2+}]_i$ fluxes induced by galectin-3

Inhibitor	Maximum inhibition (%)	Concentration
Diltiazem	40	200 μM
Nifedipine	30	100 μM
Prenylamine	10	50 μM
Pertussis toxin	0	150 ng/ml
Cholera toxin	100	50 ng/ml

Jurkat cells (10^6) were treated at 37°C for the periods given in Section 2 before measurement of $[Ca^{2+}]_i$ responses after addition of galectin-3.

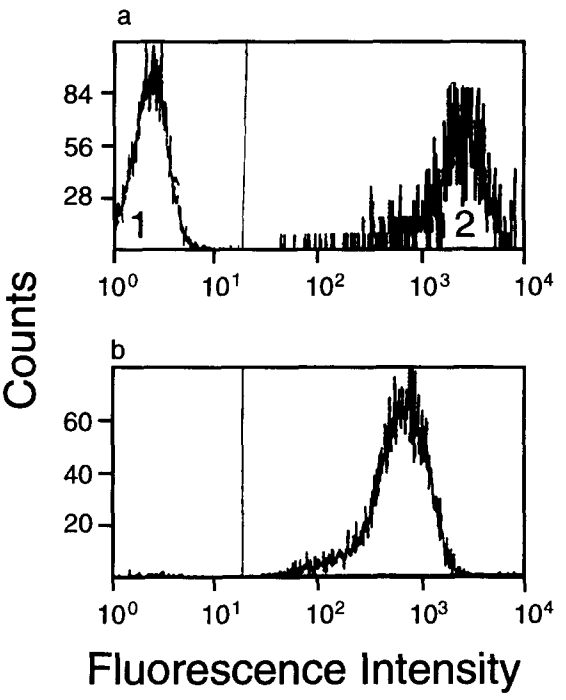

Fig. 1. Flow cytometric analysis of antigen expression on Jurkat cells. Cells were reacted with primary monoclonal antibodies followed by FITC conjugates of secondary antibodies. (a) Rat monoclonal galectin-3 antibody followed by FITC-anti rat Ig. The results of two separate analyses in which staining was performed before (line 1) or after (line 2) exposure of the cells to galectin-3 are shown superimposed; (b) mouse monoclonal 4F2 antibody followed by FITC-anti mouse Ig. Relative fluorescence intensity is shown on the abscissa and cell number on the ordinate. The vertical line in each profile indicates the position at which the negative cut-off cursor was positioned.

3.2. Jurkat cells express surface receptors for galectin-3

When viable Jurkat cells were treated for 30 min at 37°C with exogenous galectin-3 (30 μg/ml), washed and stained with the M3/38 monoclonal antibody to reveal surface bound lectin, over 99% of the cells were intensely labelled (Fig. 1a, line 2). Binding of the lectin to the cells was blocked completely by 0.2 M lactose or thiodigalactoside (results not shown), two inhibitory sugars of galectin-3 binding to model glycoprotein ligands [6].

We assessed next whether CD98 antigen was one of the surface components of Jurkat cells recognized by galectin-3, as it is in macrophages [11]. Jurkat cells are known to produce CD98 [22,23] and also to express CD98 at the cell surface as shown by flow cytometry with 4F2 monoclonal antibody (Fig. 1b). Lysates of [^{35}S]methionine-labelled cells were passed through a galectin-3-Sepharose affinity column and the bound fraction was eluted with lactose. Immunoprecipitation of the lactose eluate with the 4F2 monoclonal antibody showed the specific polypeptide pattern expected for CD98 antigen (Fig. 3). The ~85 kDa heavy chain and the ~40 kDa light chain

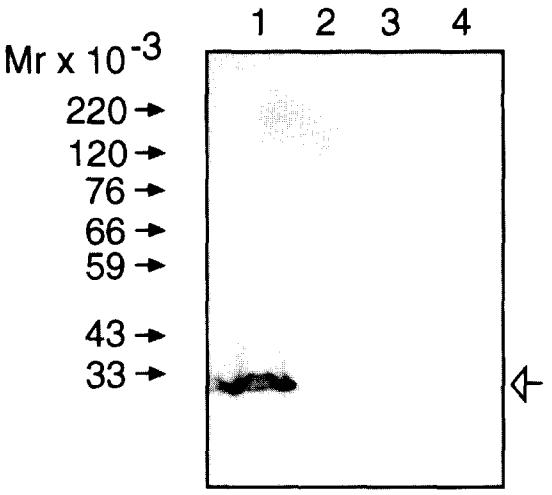

Fig. 2. Jurkat T-cells do not express galectin-3. Human THP-1 cells (10^6) treated for 3 days with TPA, or Jurkat T-cells (10^6–10^7 cells) were dissolved in reducing Laemmli buffer, subjected to SDS-PAGE followed by Western blotting with galectin-3 specific monoclonal antibody. Note expression of galectin-3 (arrowhead) in THP-1 cells treated with TPA (track 1) and no expression in 10^6 (track 2), 5×10^6 (track 3) or 10^7 (track 4) Jurkat cells.

of CD98 were separated in reducing SDS-PAGE, and in non-reducing gels the disulphide-bonded heterodimer of ~125 kDa was obtained. Other glycoprotein components of the bound fraction have not yet been identified, but do not include CD11b/CD18 (Fig. 3).

3.3. Galectin-3-induced increase in free cytoplasmic Ca^{2+}

Jurkat T-cells loaded with the fluorescent Ca^{2+} indicator Indo-1 and suspended in Ca^{2+}-containing medium showed a striking increase of $[Ca^{2+}]_i$ following addition of galectin-3 (Fig. 4A). The response was sustained and concentration dependent with a half-maximal response at 30 μg/ml of galectin-3 (approx. 1 μM). It clearly required binding of the CRD to cell surface carbohydrates since the $[Ca^{2+}]_i$ response was completely blocked by 200 mM lactose (Fig. 4B) or thiodigalactoside (result not shown). Further, the increase in $[Ca^{2+}]_i$ in cells treated with galectin-3 was due to an influx of extracellular Ca^{2+}, and not a release from intracellular stores, as no $[Ca^{2+}]_i$ increase was detected in Ca^{2+}-free buffers (Fig. 4C).

At sub-micromolar concentrations, galectin-3 appears to be monomeric. At higher concentrations chemical and enzymic cross-linking experiments [18,24] show that galectin-3 forms dimers and possibly higher oligomers and behaves as a multivalent lectin, binding to multiglycosylated ligands with positive cooperativity, and showing haemagglutinating activity. Therefore, we tested the effect of polyclonal cross-linking antibodies on the $[Ca^{2+}]_i$ response of cells treated with sub-micromolar concentrations of galectin-3 (20 μg/ml) However, addition of rabbit polyclonal antibodies directed against N-terminal or C-terminal domain epitopes of galectin-3 at dilutions varying from 1:1000 to 1:10 did not induce a $[Ca^{2+}]_i$ flux greater than that elicited (Fig. 4A) by 20 μg/ml of galectin-3 alone (results not shown). We conclude that cross-linking of cell surface ligands by galectin-3 was not necessary for signalling.

3.4. Specificity of galectin-3-induced $[Ca^{2+}]_i$ fluxes

Several plant lectins are well known to activate lymphocytes, raising the question of the specificity of the galectin 3 response in Jurkat cells. Therefore, we used two plant lectins TCA [19] and GSL-IB4 [25], that have overlapping carbohy-

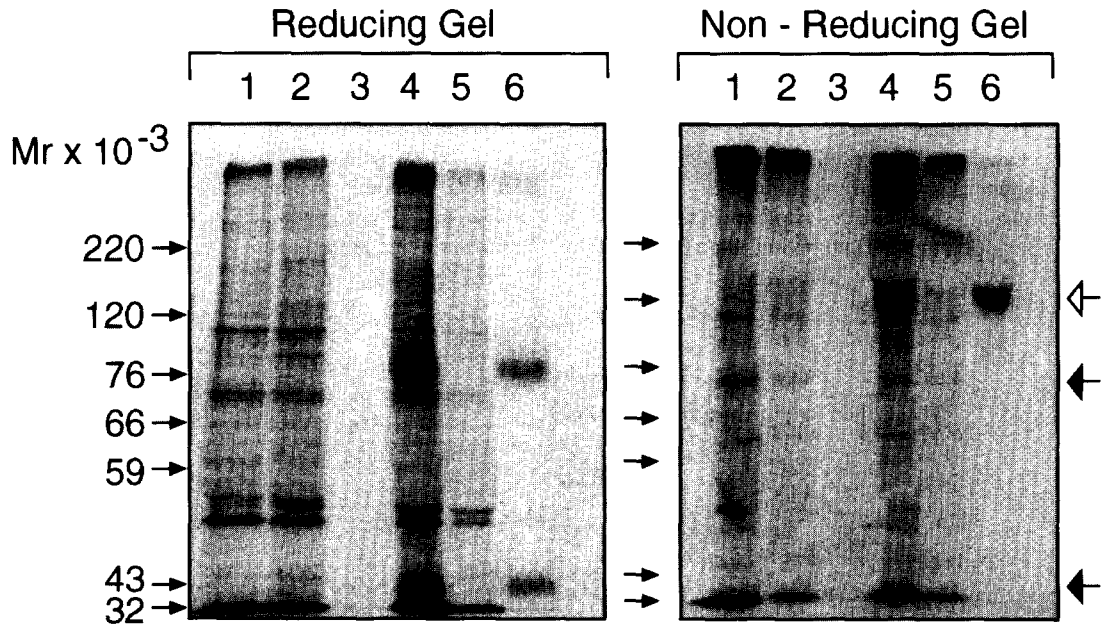

Fig. 3. CD98 (4F2 antigen) of the Jurkat T-cell line binds galectin-3. [^{35}S]Methionine-labelled cells were lysed and fractionated on a galectin-3-Sepharose affinity column. Equal portions of total lysate (tracks 1,4) unbound (tracks 2,5) and bound (tracks 3,6) fractions were immunoprecipitated with monoclonal antibodies against human CD11b/CD18 (tracks 1–3) followed by 4F2 monoclonal antibody against human CD98 heavy chain (tracks 4–6). Immune complexes were separated by SDS-PAGE under reducing or non-reducing conditions and proteins located by fluorography. The migration of heterodimeric CD98 (open arrowhead), its CD98 heavy and light chains (closed arrowheads) and molecular weight markers (arrows) is indicated.

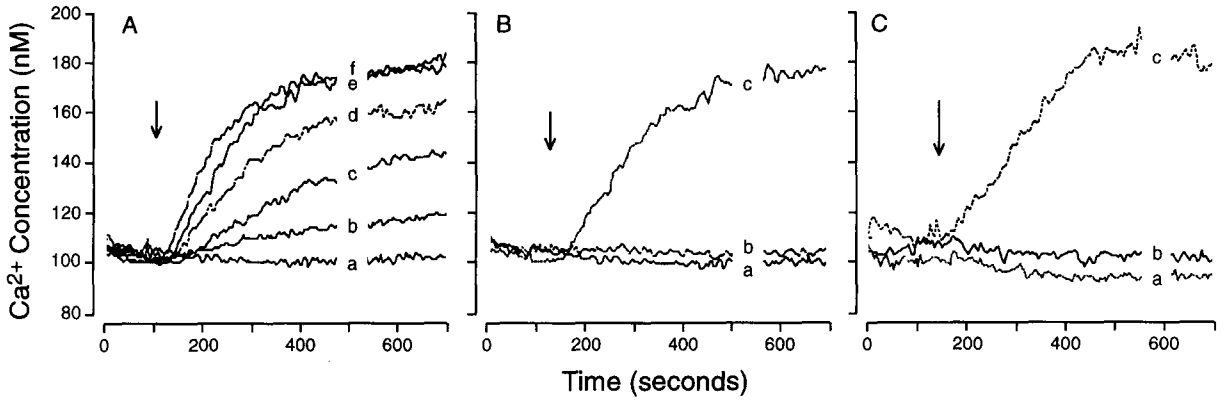

Fig. 4. Galectin-3-induced increases in cytosolic $[Ca^{2+}]_i$ in Jurkat cells. Cells suspended in buffer containing 1 mM $CaCl_2$ were loaded with indo 1-AM and base-line intracellular Ca^{2+} levels were recorded fluorimetrically before addition (arrows) of galectin-3. (A) Galectin-3 concentrations: 0 (a), 20 (b), 30 (c), 40 (d), 50 (e) and 60 (f) μg/ml, respectively, in Ca^{2+}-containing buffer; (B) galectin-3 (40 μg/ml) was added alone (c) or with 0.2 M lactose (b) in Ca^{2+}-containing buffer. Control without lectin is shown (a). (C) Galectin-3 (40 μg/ml) was added to cells suspended in buffer with (c) or without (b) Ca^{2+}. Control without lectin in Ca^{2+} containing buffer is indicated (a). Calibration was made by Triton X-100 release of total cytosolic Ca^{2+}. Traces were representative of at least three separate experiments.

drate binding specificities with galectin-3. GSL-IB4 requires α-galactosyl terminal residues as in the human B blood group epitope to which galectin-3 also binds with high affinity [6], and TCA binds to branched complex-type *N*-glycans with terminal *N*-acetyllactosamine units [19]. Flow cytometric experiments showed that Jurkat cells carry cell surface receptors for GSL-B4 as well as TCA (results not shown). Treatment of Indo 1-AM-loaded Jurkat cells with GSL-IB4 produced a slow $[Ca^{2+}]_i$ response (Fig. 5A) but only at very high concentrations (half maximal response 150 μg/ml). TCA, which is a known T-cell mitogen [19], triggered a $[Ca^{2+}]_i$ increase at concentrations 10-fold less than required for galectin-3 (Fig. 5B). In both cases, the $[Ca^{2+}]_i$ response required extracellular Ca^{2+} and was blocked completely in Ca^{2+}-free buffers (results not shown).

3.5. *Inhibitor studies*

The galectin-3-induced response in $[Ca^{2+}]_i$ was not sensitive to voltage-gated Ca^{2+}-channel blockers such as diltiazem or nifedipine nor to prenylamine (Table 1), a blocker of an ATP-dependent Ca^{2+} channel [26], Pertussis toxin had no effect even at very high concentration whereas cholera toxin blocked the $[Ca^{2+}]_i$ response at concentrations lower than 50 ng/ml (Table 1). Cholera toxin inhibits early events in T-cells following TCR activation, presumably by effects on a pertussis toxin-insensitive G-protein [27]. Further work is required to decide whether cholera toxin blockade of the galectin-3-induced $[Ca^{2+}]_i$ response occurs similarly.

4. Concluding remarks

We present data that binding of cell surface glycoproteins by galectin-3 leads to increased $[Ca^{2+}]_i$ in human Jurkat T cells. Several plant lectins, as well as antibodies to certain T cell surface antigens, can stimulate increases in $[Ca^{2+}]_i$. These responses are biphasic, consisting of an initial rapid transient phase followed by a fall in $[Ca^{2+}]_i$ to a sustained plateau level. The transient phase increase in $[Ca^{2+}]_i$ is due to Ca^{2+}-mobilisation from intracellular stores, triggered by inositol 1,4,5-triphosphate (IP_3) formation, whereas the sustained increase in $[Ca^{2+}]_i$ results from Ca^{2+} influx through plasma membrane channels [28]. The nature of the extracellular $[Ca^{2+}]_o$-dependent changes in $[Ca^{2+}]_i$ are not presently understood and the calcium channels in the leukocyte plasma membrane that mediate $[Ca^{2+}]_o$ entry have yet to be isolated. Genetic analysis suggests many gene products are implicated [29].

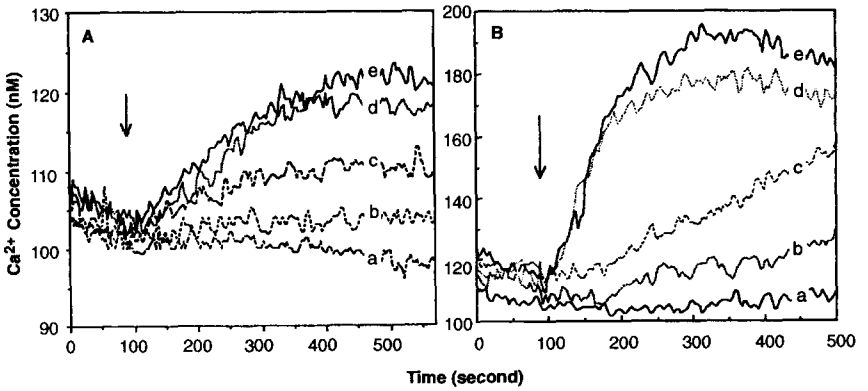

Fig. 5. Effects of plant lectins GSL-IB4 (A) and TCA (B) on cytosolic $[Ca^{2+}]_i$ in Jurkat T-cells. Indo 1-AM loaded cells were treated with lectins and cytosolic $[Ca^{2+}]_i$ levels were monitored as described in Fig. 4. (A) Concentrations were 0, 50, 100, 150 and 175 μg/ml for a–e, respectively. (B) Concentrations were 0, 2, 3, 5 μg/ml and 7.5 μg/ml for a–e, respectively.

The results of our studies with galectin-3 lead to the conclusion that this lectin stimulates Ca^{2+}-channel activity in plasma membranes. The pattern of effects of a small panel of drugs on the galectin-3-induced $[Ca^{2+}]_i$ response is identical to their effects [30] on Ca^{2+} influx triggered by antibody binding to the TCR in Jurkat cells. Unfortunately, surface membrane Ca^{2+}-channel molecules are not well characterised and specific high affinity blockers of their activities are not yet available to define precisely the target of galectin-3. It seems likely, however, that some of these surface membrane components will carry carbohydrate groups recognized by the lectin, and their ligation may be sufficient to modulate channel activity.

Interestingly, the plant lectin GSL-IB4, that binds well to Jurkat cells, induced a slow and reduced Ca^{2+} response compared with galectin 3. Although GSL-IB4 and galectin-3 show similar carbohydrate-binding specificities [6,25], there are differences in detail and it is possible that Jurkat cell surface glycoproteins exist that show higher affinity for binding to galectin-3 compared to GSL-IB4. Characterization of these glycoproteins will be of interest to identify components likely to be involved in the galectin-3-induced increase of $[Ca^{2+}]_i$ in Jurkat cells.

Acknowledgements: We thank Chris Atkins for FACS analysis and Richard Windsor for advice on Ca^{2+}-measurement. S.D. was supported by a Wellcome Trust Research Fellowship.

References

[1] Ho, M.-K. and Springer, T.A. (1982) J. Immunol. 128, 1221–1228.
[2] Hughes, R.C. (1994) Glycobiology 4, 5–12.
[3] Barondes, S.H., Cooper, D.N.W., Gitt, M.A. and Leffler, H. (1994) J. Biol. Chem. 269, 20807–20810.
[4] Sato, S., Burdett, I. and Hughes, R.C. (1993) Exp. Cell Res. 207, 8–18.
[5] Sato, S. and Hughes, R.C. (1994) J. Biol. Chem. 269, 4424–4430.
[6] Sato, S. and Hughes, R.C. (1992) J. Biol. Chem. 267, 6983–6990.
[7] Zuberi, R.I., Frigeri, L.G. and Liu, F.-T. (1994) Cell. Immunol. 156, 1–12.
[8] Frigeri, L.G., Zuberi, R.I. and Liu, F.-T. (1993) Biochemistry 32, 7644–7649.
[9] Yamaoka, A., Kuwabara, I., Frogeri, L.G. and Liu, F.-T. (1995) J. Immunol. 154, 3479–3487.
[10] Jeng, K.C., Frigeri, L.G. and Liu, F.-T. (1994) Immunol. Lett. 42, 113–116.
[11] Dong, S. and Hughes, R.C. (1995) Glycoconj. J. 12, 548.
[12] Springer, T.A., Galfre, G., Secher, D.S. and Milstein, C. (1979) Eur. J. Immunol. 9, 301–306.
[13] Haynes, B.F., Hemler, M.E., Hann, D.L., Eisenbarth, G.S., Shelhamer, J., Mostowski, H.S., Strominger, J.L. and Fauci, A.S. (1981) J. Immunol. 126, 1409–1414.
[14] Hemler, M.E. and Springer, T.A. (1982) J. Immunol. 129, 623–628.
[15] Bron, C., Rovsseaux, M., Spiazzi, A.-L. and MacDonald, H.R. (1986) J. Immunol. 137, 397–399.
[16] Parmacek, M.S., Karpinski, B.A., Gottesdiener, K.M., Thompson, C.B. and Leiden, J.M. (1989) Nucleic Acids Res. 17, 1915–1931.
[17] Foddy, L. Stamatoglou, S.C. and Hughes, R.C. (1990) J. Cell Sci. 97, 139–148.
[18] Mehul, B., Bawumia, S., Martin, S.R. and Hughes, R.C. (1994) J. Biol. Chem. 269, 18250–18258.
[19] Sato, S., Animashaun, T. and Hughes, R.C. (1991) J. Biol. Chem. 266, 11485–11494.
[20] Laemmli, U.K. (1970) Nature 227, 680–685.
[21] Bijsterbosch, M.K., Rigley, K.P. and Klaus, G.G.B. (1986) Biochem. Biophys. Res. Commun. 137, 500–506.
[22] Spagnoli, G.C., Ausiello, C., Palma, C., Bellone, G., Ippoliti, G., Letarte, M. and Malavasi, F. (1991) Cell Immunol. 136, 208–218.
[23] Friedman, A.W., Diaz, L.A., Moore, S., Schaller, J. and Fox, D.A. (1994) Cell. Immunol. 154, 253–262.
[24] Mehul, B., Bawumia, S. and Hughes, R.C. (1995) FEBS Lett. 360, 160–164.
[25] Petryniak, J., Huard, T.K. and Goldstein, I.J. (1992) Eur. J. Biochem. 206, 197–207.
[26] Popper, L.D. and Batra, S. (1993) Cell Calcium 14, 209–218.
[27] Imboden, J.B., Shoback, D., Pattison, S. and Stobo, J.D. (1986) Proc. Nat. Acad. Sci. USA 83, 5673–5677.
[28] Gardner, P. (1989) Cell 59, 15–20.
[29] Serafini, A.T., Lewis, R.S., Clipstone, N.A., Bram, R.J., Fanger, C., Fiering, S., Herzenberg, L.A. and Crabtree, G.R. (1995) Immunity 3, 239–250.
[30] Sei, Y., Takemura, M., Guskovsky, F., Skolnick, P. and Basile, A. (1995) Exp. Cell Res. 216, 222–231.