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Ion channels activated by specific Ti or T3 antibodies in plasma membranes of human T cells

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T lymphocytes are activated to proliferate via a surface membrane receptor recognizing the antigen/major histocompatibility complex. This membrane component is comprised of at least five polypeptide subunits, collectively termed the Ti-T3 receptor complex. A transient increase in cytosolic free calcium occurs as an early event in the T-cell activation process and is necessary for induction of the endogenous IL-2 and certain other genes. Monoclonal antibodies specific to epitopes of either the Ti or the T3 components were shown to be effective agonists, also leading to such transient rises in cytosolic free calcium and activating the lymphocytes. Here we show, using micropipette-supported bilayers formed from membranes of the human T-cell line REX, that Ti- or T3-specific antibodies cause opening of ligand gated ion channels. Both types of specific antibodies yielded similar histograms of conductance amplitudes which show a channel with a conductance of 2-3 pS in symmetrical 100 mM $CaCl_2$ solutions. These channels allow the passage of calcium and barium ions and are blocked by lanthanum ions, suggesting that they are specific for calcium. We propose that these channels, by allowing the entry of external calcium, may account for a large fraction of the rise in intracellular calcium observed upon triggering of the Ti-T3 receptor.

Key words: T lymphocytes/Ti-T3 receptor complex/monoclonal antibodies/receptor gated channels/calcium ions

Introduction

Human T lymphocytes can be activated to proliferate via an interleukin-2-dependent autocrine pathway by antigen binding to specific receptors (Meuer *et al.*, 1984a). The latter cell surface component which recognizes the antigen in conjunction with the appropriate MHC elements is a multimeric protein complex. It consists of the putative antigen recognition element Ti, a disulfide-linked polymorphic heterodimer, with which a group of at least three polypeptides designated T3 is strongly associated (Reinherz *et al.*, 1982, 1983; Acuto *et al.*, 1983; Meuer *et al.*, 1983a, 1984b; Oettgen *et al.*, 1984; Borst *et al.*, 1985). Monoclonal antibodies specific to the Ti or T3 can mimic the cellular activation induced by antigen (Meuer *et al.*, 1983b, 1984b). An early event detected in human or murine T cells following stimulation by, for example, Ti- or T3-specific monoclonal antibodies, is a transient increase in their cytosolic free Ca^{2+} concentration, $[Ca^{2+}]_i$ (Weiss *et al.*, 1984a,b; Oettgen *et al.*, 1985; Shapiro *et al.*, 1985). This and the finding that artificially raising $[Ca^{2+}]_i$ by ionophores leads to mitogenesis, supported the second messenger role of these ions (Tsien *et al.*, 1982). This raises the question of whether the increase in $[Ca^{2+}]_i$ occurs by influx from extracellular medium or is being released from intracellular stores. Several lines of evidence support the former possibility though release from cellular depots is most probably also taking place (Oettgen *et al.*, 1985; Imboden and Stobo, 1985). The opening of ion channels in the plasma membrane has been proposed as the mechanism for influx of Ca^{2+} down its marked concentration gradient (Alcover *et al.*, 1986). The mitogenic lectin phytohaemagglutinin (PHA) has been shown to open calcium channels in the cell membrane of T lymphocytes (Kuno *et al.*, 1986) and similar channels were induced by inositoltriphosphate (IP3) added to the intracellular side of such membranes (Kuno and Gardner, 1987). To directly examine the possibility that the interaction of specific monoclonal antibodies with Ti or T3 causes the opening of ion channels, we investigated the conductance properties of micropipette-supported bilayers prepared from plasma membranes of the human T-cell line REX (Acuto *et al.*, 1984) as effected by antibodies specific to these membrane components.

Results

Plasma membranes of the human T-cell line REX together with exogenous phospholipids were used to form bilayers at the tip of glass micropipettes. These bilayers were stable and did not show channel activity in the absence of added antibodies at any transmembrane potential in the range used (± 100 mV). This indicates that under these experimental conditions no voltage-dependent channel can be observed. Seven different monoclonal antibodies were now individually examined for their capacity to induce channel activity in these micropipette-supported bilayers (Table I). The three monoclonal antibodies that induced channel activity are either specific for epitopes of the Ti receptor of the REX line or of the T3 complex. Figures 1 and 2 illustrate record-

Table I. Characteristics of monoclonal antibodies tested for induction of channel activity in T cell bilayers

Monoclonal antibodies	Isotype	Specificity	Conducance change[a]
RW2-8C8	IgG1	Anti-T3	+
2Ad2	IgM	Anti-T3	+
5REX9H5	IgG2a	Anti-Ti (R)[b]	+
2ST812F10	IgG1	Anti-Ti (1RR)[b]	−
RW2-6H8	IgG1	Anti-Ti (1RR)[b]	−
19Thy5D7	IgG2a	Anti-T4	−
18T3A9	IgG1	Anti-T4	−

[a]+ and − denote the presence or absence of antibody-induced conductance changes respectively.
[b]5REX9H5 is the anticlonotypic monoclonal antibody for the REX tumor line [anti-Ti (R)] (Acuto *et al.*, 1983) whereas 2ST812F10 and RW2-6H8 are irrelevant anti-clonotypes [anti-Ti (1RR)] (Bensusan *et al.*, 1984). RW2-8C8 and 2Ad2 are anti-T3 antibodies (Reinherz *et al.*, 1982). 19Thy5D7 and 18T3A9 are anti-T4 monoclonal antibodies (Sayre and Reinherz, 1985).

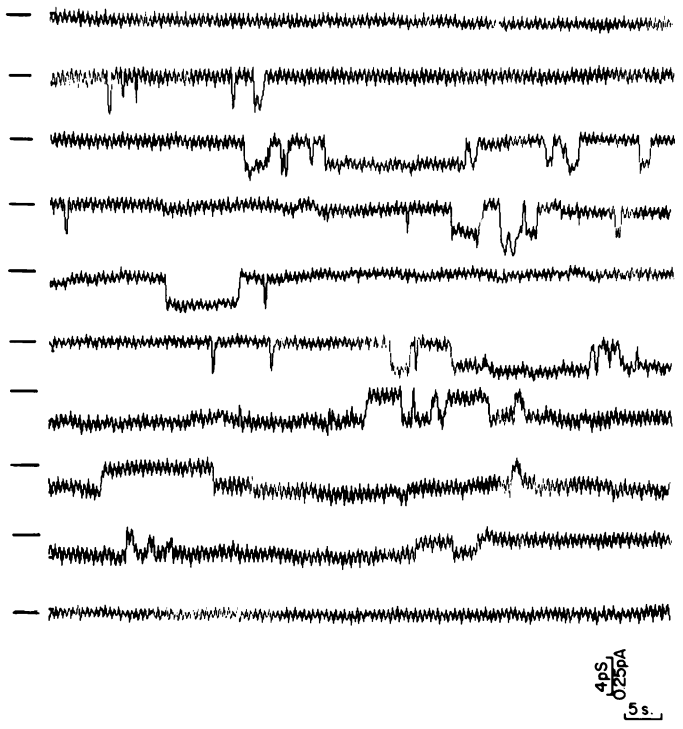

Fig. 1. Channel activity induced by the T3-specific monoclonal antibody RW2-8C8. The uppermost trace in the figure shows a partial record of the baseline current in an experiment carried out at −60 mV (pipette interior negative). The current signal has been low pass filtered at 120 Hz and digitized at 1000 points/s. The next eight traces (all of them except the last one) represent a continuous record starting 2 min after adding the anti-T3 antibody to the well (20 μg/ml final concentration). Channel opening appears as a downward deflection in the current record, representing charge flowing into the pipette. The last trace in this figure was taken 2 min after adding La^{3+} ions (100 μM final concentration) to the well.

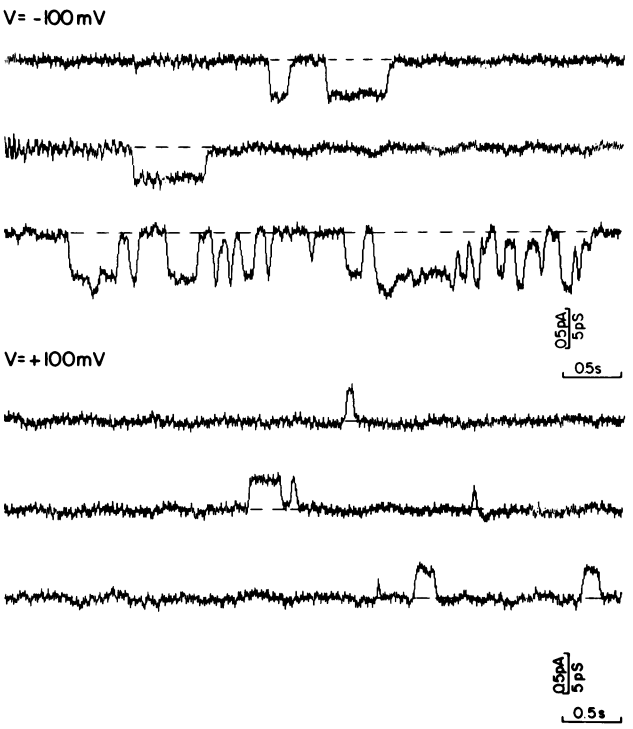

Fig. 2. Channel activity induced by the anti-T3 antibody RW2-8C8 at different membrane potentials. The antibody was added with the membrane clamped at −100 mV (upper half). The traces shown were chosen in order to illustrate the two types of most frequently observed events: (i) isolated events with relatively long non-conducting intermediate periods (first two traces), and (ii) burst behavior with many events separated by very short intervals. After channel activity was recorded at −100 mV, the potential was reversed and clamped at +100 mV (pipette interior positive now). Under these conditions, channel activity appeared as an upward deflection of the current trace. Data were low pass filtered at 80 Hz and digitized at 2 kHz.

ed stretches of conductance changes induced by a T3-specific antibody (clone RW2-8C8). Figure 1 shows a 15-min record of a typical experiment carried out at −60 mV (pipette interior negative with reference to the grounded well). The first stretch is the base-line current before the addition of the antibody. The next eight traces show channel activity induced by the anti-T3 antibody. The last trace in the figure was obtained after the addition of La^{3+} ions (100 μM) to the well and shows that channel activity has completely disappeared. Figure 2 illustrates the inversion of the current direction with a change in voltage polarity. Antibody-induced channel activity is observed at any voltage in the range studied (± 100 mV) but the frequency of channel opening decreases at positive pipette potentials. Since the antibodies are added to the well into which the pipette is immersed, the well may formally represent the extracellular medium while the pipette solution represents the interior of the cell. Thus, although these micropipette-supported bilayers are essentially symmetric, the probability of channel opening is higher when the pipette is clamped at a negative voltage, i.e. at potential values in the physiological polarity. Oettgen *et al.* (1985) studying another human T-cell line (leukemic line HPB-ALL), have shown that depolarization of the membrane potential reduces the antibody-induced rise in $[Ca^{2+}]_i$. Figure 3 shows conductance changes induced by a Ti specific antibody (line 5REX9H5). The traces shown in this figure were selected to illustrate the relatively infrequent appearance of more than one conductance level.

In experiments with antibodies that induced channel activity, a mean of 100 events were recorded per 10-min observation time. Histograms of single channel conductances summarizing individual experiments carried out using three different agonist-like antibodies are shown in Figure 4. Significantly, the most frequent events in all three histograms are characterized by conductance values which lie within the same range of ~2−3 pS.

Four other monoclonal antibodies examined did not induce any detectable conductance changes. These were two anti-Ti antibodies specific for different T-cell lines (clones 2ST812F10 and RW2-6H8) (Bensusan *et al.*, 1984), and two antibodies specific for T4 epitopes (clones 19Thy5D7 and 18T3A9). The fact that anti-T4 antibodies (Sayre and Reinherz, 1985)which are known to bind to REX plasma membranes, do not induce any channel activity indicates that protein aggregation in the plane of the membrane by itself is not sufficient for channel opening. The lack of response to the different antibodies used as controls also eliminates the possibility that an artifactual interaction between the antibodies and other proteins residing in the lipid bilayer is the cause of the conductance changes observed. No dependence of channel activity on the isotype of the antibodies employed in the individual experiments is observed. Both anti-T3 antibodies (RW2-8C8 and 2Ad2) induce channel opening although they are of IgG1 and IgM isotypes respectively (Reinherz *et al.*, 1982). In contrast, the anti-T4 antibodies which are of the IgG2a (19Thy5D7) and IgG1 (18T3A9) isotypes (Sayre and Reinherz, 1985) are without any effect. In the experiments reported herein, antibodies that induced channel activity did so in >50% of the trials. This is quite an acceptable ratio of positive observations.

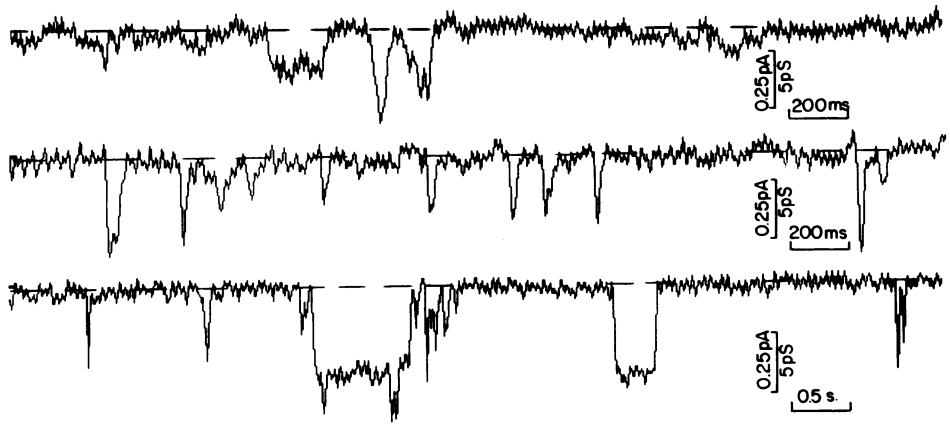

Fig. 3. Channel activity induced by the clone-specific anti-Ti antibody 5REX9H5. Traces shown were taken from three different experiments to illustrate the presence of multilevels and the higher conductance levels observed. In the three experiments the transmembrane potential was −50 mV. Data were filtered at 50 Hz and digitized at 3 kHz. Normally, most of the events detected in every experiment have a similar amplitude, in the range 2−4 pS, as shown in Figures 1 and 2. In some cases short stretches appear with higher conductance levels of up to 8−9 pS.

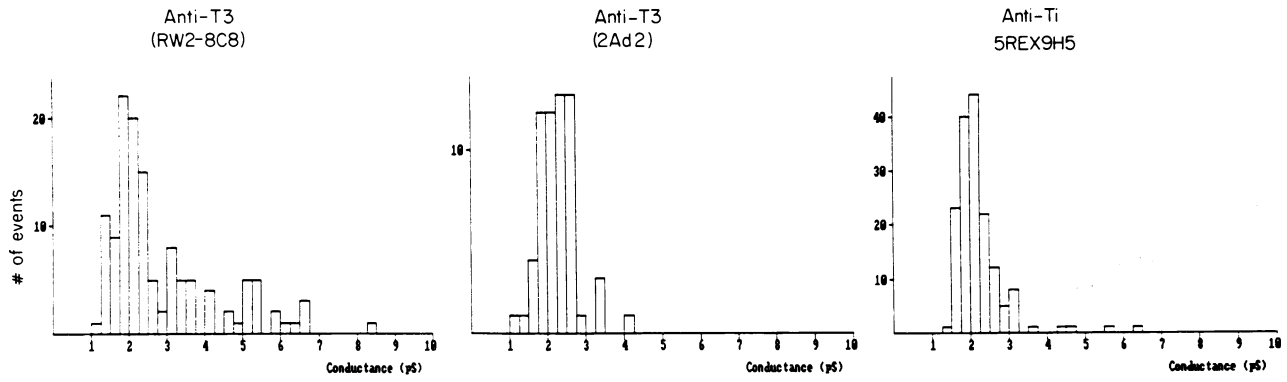

Fig. 4. Conductance histograms of the events induced by the effective monoclonal antibodies in individual experiments. Events detected during the first 10 min following addition of the respective antibody are included. In the three experiments shown clamp potential was −50 mV. Details of the experiments are as in the previous figures. The number of events analysed in each histogram is 85, 61, and 160 respectively. Although the main populations of observed events in each experiment has a conductance of 2−3 pS, there is, in some cases, a tail in the conductance histogram corresponding to events of higher conductance (see also Figure 3). The appearance of such a tail was observed in experiments with each of the effective antibodies used.

Considering the number of Ti−T3 receptor copies per cell (~30 000) (Meuer *et al.*, 1984b) and taking into account that the original membranes have been diluted ~5-fold with exogenous lipids, an average of ~4−5 receptor units per bilayer is expected. Antibodies that did not induce channel activity, failed in all independent experiments (at least five) performed with each of them.

These results clearly indicate that T3 or REX-clonotype-specific antibodies are able to induce channel activity in T-cell membranes. However, since the reconstituted bilayers are bathed in symmetrical $CaCl_2$ solutions, information concerning the charge carrier through the channels is not yet conclusive, although the blocking effect of La^{3+} suggests that the channel is selective for Ca^{2+}. To further resolve this question, several experiments were performed utilizing Ba^{2+} instead of Ca^{2+} as the cation present in the solutions. Figure 5 shows two traces of channel activity induced by the anti-T3 antibody 2Ad2, in the presence of $BaCl_2$ (50 mM) in both the well and pipette solutions. The single channel conductance observed under these conditions is ~7−8 pS, a value significantly higher than that obtained in the presence of 100 mM $CaCl_2$. This indicates that Ba^{2+} can also be the current carrier through these antibody-induced channels.

Discussion

Stimulation of T lymphocytes by anti-Ti or anti-T3 antibodies was shown to cause an early transient rise in intracellular free-calcium concentration (Weiss *et al.*, 1984a,b; Oettgen *et al.*, 1985; Shapiro *et al.*, 1985). In the present study it was shown that the same antibodies which stimulate the REX T cells, also induce channel activity in bilayers reconstituted from these very same cells' plasma membranes. In these experiments, channel activity was induced either by REX-clone-specific anti-Ti antibodies or by anti-T3 antibodies, suggesting specificity of the effect for the given Ti−T3 receptor complex. Our experimental protocol eliminates the possibility that voltage-dependent channels are being activated in the reconstituted membranes, since, in the absence of antibodies, no channel activity is observed at any transmembrane potential. The results of experiments carried out with the T4-specific antibodies also rule out the possibility that binding of antibodies to membrane components other than the Ti−T3 complex, may be the cause for the observed changes in membrane conductivity.

That the ion channels opened by Ti- or T3-specific antibodies are selective for Ca^{2+} ions is suggested by two independent ex-

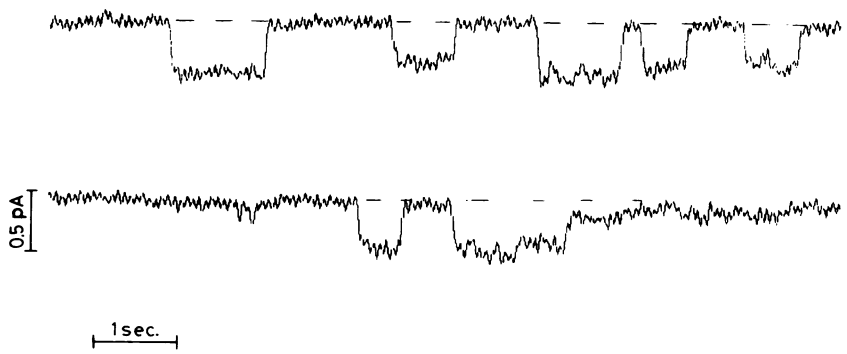

Fig. 5. Channel activity in the presence of Barium ions. The triggering agent in this example was the anti-T3 antibody 2Ad2. Solutions on both sides of the reconstituted membrane contained 50 mM $BaCl_2$ and 10 mM Tris−HCl, pH 7.5. Data were filtered at 50 Hz and digitized at 3 kHz.

perimental results. First, antibody-induced channels are blocked by La^{3+} ions. Lanthanum has been extensively used as a blocker of calcium channels (Nelson *et al.*, 1984; Suarez-Isla *et al.*, 1986). Moreover, the same concentration of La^{3+} ions used in our experiments (100 μM) has been shown to block the anti-T3 induced increase of $[Ca^{2+}]_i$ in another human T-cell line (Oettgen *et al.*, 1985). Second, the results obtained in the presence of Ba^{2+} ions also support the notion that these antibody-induced channels are calcium channels. Barium has so far been shown to cross every calcium channel examined with higher conductance than calcium itself (Nelson, 1986). Indeed in our experiments we observe that the conductance of antibody-induced channels is higher with Ba^{2+} than with Ca^{2+} ions as charge carriers. Barium was also shown to block K^+ channels, if these are present, and to prevent the opening of Ca-dependent K^+ channels known to be present in membranes of T lymphocytes (Chandy *et al.*, 1984, 1985). The results obtained with $BaCl_2$ eliminate therefore the possibility that the channel events observed in the presence of $CaCl_2$ are due to calcium ions leaking through K^+-specific channels since these K^+ channels are expected to be blocked in the presence of barium. These findings also eliminate the alternative that Cl^- ions would be the current carriers through the antibody-induced channels. The conductance of a chloride channel would be expected to decrease in the presence of 50 mM $BaCl_2$ as compared with 100 mM $CaCl_2$. This, however, is contrary to our experimental finding. Blockage by lanthanum and permeability to barium are typical characteristics of voltage-dependent calcium channels. They are also shared by the antibody-induced channels reported in this study although these channels cannot be induced to open by voltage in the absence of the antibodies.

The ion channels detected in our experiments could account for the transient increase in $[Ca^{2+}]_i$ following specific antigen stimulation of the Ti−T3 complex. This is supported by the fact that the same antibodies specific for T3 or Ti that induce the La^{3+}-blockable channel activity in bilayers formed from REX membranes also cause the $[Ca^{2+}]_i$ rise in intact REX cells (Alcover *et al.*, 1986).

That monoclonal antibodies against idiotypic (Ti) or monomorphic (T3) components of the antigen/MHC receptor induce calcium ion influx does not necessarily imply that a putative ion channel is within the receptor complex itself. It has recently been demonstrated (Alcover *et al.*, 1986) that antibodies directed at the 50-kd T11 structure cause an increase in $[Ca^{2+}]_i$ and that this pathway is functional in precursor T-lineage cells which lack the surface Ti−T3 complex. This suggests that there may be a plasma membrane Ca^{2+} channel functionally or physically linked to the T11 structure, or alternatively, that there is a common set of related T11 and Ti−T3-associated channels. Documentation of channels conductance initiated upon T11 triggering in this bilayer system will be of importance. Moreover, comparison of conductance characteristics will show whether related or distinct channel types are involved.

Recently, Kuno *et al.* (1986) have obtained evidence using the whole-cell patch clamp technique showing that in cloned human helper T lymphocytes, the mitogenic lectin PHA induces the opening of voltage-independent Ca^{2+} channels. They have also shown (Kuno and Gardner, 1987) that similar channel activity can be induced in inside-out membrane patches excised from the same cloned cells, by addition of inositoltriphosphate (IP3) to the intracellular side of the cell membrane. These channels have a conductance of ~7 pS when Ba^{2+} ions are the charge carriers, a value similar to the one obtained by us for the antibody-induced channels in the presence of barium. Whether the Ca^{2+} channel observed by Kuno *et al.* (1986) is the same one reported in this paper remains to be established. The triggering agents are indeed different. However, IP3 is a soluble product of the hydrolysis of phosphatidylinositol bisphosphate (PIP2) (Berridge, 1984). Specific antigen or antibody stimulation of T lymphocytes also leads to hydrolysis of PIP2 (Imboden and Stobo, 1985). If both channels, the one reported here, induced by specific antibodies to the Ti−T3 receptor complex, and the IP3-induced channel reported by Kuno and Gardner (1987) are the same or related, the following interesting situation is encountered; antigen binding to the T-cell receptor induces direct opening of calcium channels and at the same time also causes production of IP3 which in turn acts on the channel to increase its opening probability or will cause more channels to open. Such a cascade could potentiate the effects of antigen binding in increasing and maintaining an increased $[Ca^{2+}]_i$. This could provide a common calcium channel to be utilized by antigen and also by mitogens that do not react directly with the T-cell receptor complex.

The large increase in $[Ca^{2+}]_i$ that follows T cell antigen/MHC receptor triggering is required for induction of the endogenous IL-2 gene in helper and certain cytotoxic T lymphocytes. Thus, the ability to rapidly alter the intracellular ionic milieu through membrane channels appears to be a necessary feature for the T-cell growth stimulation processes. However, the additional role of intracellular or extracellular Ca^{2+} stores in the physiology of these cells is yet to be fully appreciated.

Ligand-operated ion channels have also been observed with other cells of the immune system such as the macrophage Fc receptor for IgG2b and IgG1 (Young *et al.*, 1983) and the mast cell Fc-receptor-activated channels (Mazurek *et al.*, 1984; Cor-

cia *et al.* (1986). Further characterization of these systems will hopefully resolve their structural features, ionic specificity and their mechanisms of action.

Materials and methods

Plasma membranes of the human T-cell tumor line REX were prepared as follows. Cells were lysed in minimal essential medium under pressure (500 psi for 15 min) in a Parr 4635 cell disruption bomb (Parr Instruments, Moline, IL) and the resulting homogenate centrifuged twice (15 min at 450 *g* and 15 min at 4000 *g*), discarding the pellets each time. The supernatant from the second sedimentation was centrifuged for 1 h at 17 000 *g* and the resulting pellet homogenized in 10 mM Tris−HCl buffer, pH 7.8, 0.2 mM $MgCl_2$, 1 mM PMSF and stored frozen at −70°C. Aliquots of the isolated REX membranes (50 μl each, containing membranes of $\sim 2.5 \times 10^7$ cells) were diluted with 300 μl of the experimental buffer (100 mM $CaCl_2$ or 50 mM $BaCl_2$, 5 mM Tris−HCl, pH 7.5) and dialysed for at least 4 h against the same experimental buffer.

A thin layer of lipids was prepared by dissolving 0.87 mg of soybean lecithin together with 46 μg of cholesterol (19:1 weight ratio) in 260 μl of 2:1 chloroform/methanol in a round 100-ml glass flask and drying it under a stream of N_2. The soybean lecithin (Asolectin, Type IV-S, Sigma Co.) was previously purified as described by Kagawa and Racker (1971). Liposomes were prepared by using the dialysed solution of membranes to resuspend the dried lipid layer, and sonicating this suspension for 5 min.

Micropipettes pulled from borosilicate glass capillaries by the two-step method (Hamill *et al.*, 1981) were filled with one of the above experimental solutions (either 100 mM $CaCl_2$ or 50 mM $BaCl_2$) and their tip (~ 1 μm diameter) was immersed in a well of a microtiter plate containing 75 μl of the same solution. The resistance of the micropipettes was monitored continuously (20−50 MΩ). One hundred microliters of the liposome suspension were then added to this well and allowed to equilibrate for ~ 15 min and form a monolayer at the water−air interface. At this point, the pipette was moved out and then back through the monolayer into the solution by a micromanipulator to produce a bilayer at its tip (Coronado and Latorre, 1983). This process was monitored by following the increase in pipette resistance. Once the bilayer was formed, its stability was monitored for 10 min at the potential which was chosen for the experiment (usually in the range of 50−100 mV, pipette interior negative). Maintained resistance of this micropipette-supported bilayer assured its stability and the absence of intrinsic conductance fluctuations induced by the potential itself. The bilayers used in this study had resistances in the range 30−200 GΩ. After this period of inspection which ascertained that no voltage-dependent channels were detected in the formed bilayer, the different antibodies were then added to the well and the currents flowing through the bilayer were measured with an EPC-5 patch-clamp system (List Electronics, FRG). Currents were monitored and recorded on an FM magnetic tape for a continuous period of at least 10 min. Each of the monoclonal antibodies was used either in ascites form or as protein-A-purified form in the case of antibodies of the IgG subclasses. Final concentration of antibodies in the well was ~ 20 μg/ml.

In all cases where antibody-induced channel activity was observed, $LaCl_3$ (100 μM final concentration) was added to the well at the end of the recording period to check their blocking effect. All experiments were carried out at room temperature.

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