

Signal Transduction by the CD2 Antigen in T Cells and Natural Killer Cells: Requirement for Expression of a Functional T Cell Receptor or Binding of Antibody Fc to the Fc Receptor, Fc γ RIIA (CD16)

By Louise L. Spruyt, Martin J. Glennie,* Albertus D. Beyers, and Alan F. Williams

*From the Medical Research Council Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE; and *Tenovus Research Laboratory, General Hospital, Southampton SO9 4XY, United Kingdom*

Summary

Crosslinking of CD2 antigen on T lymphocytes and natural killer (NK) cells leads to a rise in cytoplasmic-free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). However, CD2 seems unlikely to interact directly with the second messenger pathways since signaling via CD2 is poor in T cells that lack the T cell receptor (TCR) and is absent in L cells or insect cells that express CD2. In contrast, NK cells that are also TCR $^-$ can be triggered via CD2, but it is unclear as to whether the CD16 Fc receptor (FcR) may facilitate this effect. The CD16 transmembrane molecule is expressed in a complex with the zeta homodimer or the ζ/γ heterodimer and these dimers are also associated with the TCR complex. Thus, it seemed that ζ chains may provide the link between signaling on NK cells and T cells. This could be tested on TCR $^-$ cells since when CD16 is transfected into T cells it is expressed in a complex with TCR ζ homodimer or the ζ/γ heterodimer. At first, potentiation of CD2 signaling was seen on TCR $^-$ Jurkat cells expressing CD16, but this was found to be dependent on trace levels (1%) of IgG in F(ab') $_2$ antibody preparations. With pure F(ab') $_2$, the effect was lost. Signaling on a rat NK cell line was also re-examined with F(ab') $_2$ antibodies that had no IgG contamination, and again no signal transduction via CD2 was seen. We thus conclude that there is no clear evidence for potent signaling via CD2 on cells that lack a TCR complex and that TCR ζ chain expressed at the cell surface is not sufficient to potentiate signaling via CD2 as measured by an increase in $[\text{Ca}^{2+}]_i$.

CD2 is a glycoprotein of 50 kD that is expressed on thymocytes, T lymphocytes, and NK cells (1). The molecule consists of two Ig-like domains followed by a single transmembrane sequence and a COOH-terminal cytoplasmic region of 116–117 residues (2). CD2 can mediate adhesion of T cells to other cells by binding to its ligand LFA-3, which has a similar structure to CD2 (2a).

T lymphocytes can be activated in an antigen-dependent manner through the TCR/CD3 complex or in vitro in an antigen-independent manner through one of several surface molecules including CD2. Stimulation through CD2 has been referred to as the alternative activation pathway (3) and, as in the case of activation through the TCR/CD3 complex, leads to inositol phosphate and diacylglycerol mobilization, a rise in cytoplasmic-free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$)¹ (1,

2), phosphorylation of the ζ chain of the TCR/CD3 complex on tyrosine residues (4), expression of the IL-2R, IL-2 secretion, and mitogenesis (1, 2). For human and rat CD2, the cytoplasmic domain is required for signal transduction as measured by increases in $[\text{Ca}^{2+}]_i$ or IL-2 secretion (1, 2).

A number of studies have indicated a functional interrelationship between CD2 and the TCR/CD3 complex (1, 2). CD2 can synergize with the TCR by enhancing antigen-mediated IL-2 secretion in T cell hybridomas (5). In TCR/CD3 $^-$ T cell lines, crosslinking of CD2 functions poorly in inducing elevations in $[\text{Ca}^{2+}]_i$ (1, 2) or IL-2 secretion (1). CD2 is dependent on the presence of CD3 for the induction of MAP-2 kinase activity (6). CD2 does not transduce activation signals if expressed on an insect gut epithelial cell line by using the Baculovirus system (7) or on a murine L fibroblast line (8). Downmodulation of the TCR/CD3 complex by means of mAbs prevents subsequent stimulation via CD2 (9).

TCR-independent signaling via CD2 has been reported

¹ Abbreviations used in this paper: $[\text{Ca}^{2+}]_i$, cytoplasmic-free Ca^{2+} concentration; DAB, Dulbecco's A + B PBS solution; GPI, glycosyl phosphatidylinositol; PI-PLC, glycosyl-phosphatidyl inositol-specific phospholipase C; RAM, rabbit anti-mouse IgG.

to occur in NK cells. NK cells are TCR/CD3⁻, but they express the transmembrane form of the Fc receptor CD16 or FcγRIIIA, henceforth called CD16 (T/M), in a complex with ζ chain homodimers or ζ/γ heterodimers, as found in the TCR/CD3 complex (10). CD16 (T/M) mediates signal transduction on NK cells since its interaction with immune complexes or mAbs induces increases in [Ca²⁺]_i, mobilization of inositol phosphates (11), and tyrosine phosphorylation of the ζ chain (12). CD16 can also be expressed in a form anchored by a glycosyl-phosphatidyl inositol anchor, CD16 (GPI), and this form does not mediate signal transduction (13).

There have been conflicting reports about the signal transduction ability of CD2 on CD3⁻/CD16⁺ NK cells. Cross-linking of CD2 on the surface of a rat NK-like cell line leads to the generation of inositol phosphate (14). Anti-CD2 mAb use in the form of ascites caused NK cells to lyse FcR-expressing NK cell-resistant targets (15). In NK cells stimulated with an (anti-CD2 × anti-DNP) conjugate, induction of cytotoxicity via CD2 was shown to be dependent on interplay with CD16 (16). Anti-CD2 IgG of the IgG3 isotype caused a rise in [Ca²⁺]_i and an increase in cytolytic activity in cultured NK cells. These effects were not seen when the anti-CD2 mAb was used in the form of F(ab')₂ fragments, demonstrating that activation of the lytic response in NK cells requires cocrosslinking of CD2 and CD16 (T/M) (17).

The common element found in the TCR and CD16 (T/M) signal transduction complexes of T cells and NK cells are the disulphide-linked dimers ζ/ζ, ζ/γ, and γ/γ. This suggested that these dimers are crucial for signaling via the TCR and CD16 (T/M). We hypothesized that expression of CD16 (T/M) with its associated dimers might restore signaling via CD2 in TCR/CD3⁻ Jurkat cells and tested this hypothesis by transfection of CD16 (T/M) cDNA into such cells.

Materials and Methods

Cell Lines. The TCR⁻ Jurkat cell line J.RT3-T3.5 (18) and the TCR⁺ Jurkat cell line E6-1 (19) were both obtained from Arthur Weiss (UCSF, CA). The E6-1 Jurkat cells were subcloned and a clone designated J5 was selected for further use.

The rat NK cell line RNK-16 was obtained from John Imboden (UCSF, CA) (14). This cell line was contaminated with mycoplasma and was treated with 40 μg/10 ml BM-CYCLIN (Boehringer Mannheim, Sussex, UK). The rat cell line A181 was obtained from Leon Cobb (MRC Radiobiology Unit, Oxon) (L.L. Spruyt and M.J. Glennie, unpublished results). This line recently arose in a Fisher rat and was grown by passage through Fisher rats. It was used after the third passage and resembled the line RNK-16 in that it was CD2⁺, CD3⁻, and NKR-P1⁺. All cell lines were grown in medium containing RPMI 1640 supplemented with 10% heat-inactivated FCS, 50 μM 2-ME, and antibiotics.

Monoclonal Antibodies. The following mAbs were used: mouse anti-human CD3, OKT3 (IgG2a) (20); mouse anti-human CD2, OKT11 (IgG1) (21); GT2 (IgG1), which was kindly donated by Doreen Cantrell (ICRF, London) (22); mouse anti-human CD16, 3G8 (IgG1), which was kindly donated by David Segal (NIH, Bethesda, MD) (23); mouse anti-rat CD2, OX-34 (IgG2a) (2); mouse anti-mouse TCR-β, F23.1 (IgG2a) (24); mouse anti-rat

CD3, 1F4 (IgM), which was kindly donated by Tosiya Tanaka (Tohoku University, Japan) (25); mouse anti-human 'A' erythrocytes, W6/1 (IgM) (not published); mouse anti-human C3b inactivator, OX-21 (IgG1). The mouse anti-rat mAb 3.2.3 (IgG1), which was obtained from Serotec (Kidlington UK), recognizes an antigen, NKR-P1, on the surface of rat NK cells (26). Bispecific mouse anti-human CD2 (GT2 × OKT11) F(ab')₂ comprising one OKT11 Fab'γ arm and one GT2 Fab'γ arm was prepared by joining the individual antibody Fab'γ fragments via a stable thioether linkage as previously described (27). These F(ab')₂ fragments were then purified over a highly specific sheep anti-mouse Fc immunosorbent column.

Expression Vectors for CD16 T/M and CD16 GPI. cDNA clones FcγRIII-1 (CD16 GPI) and FcγRIII-2 (CD16 T/M) were obtained from Jeffrey Ravetch (Memorial Sloan-Kettering Cancer Center, New York) (28) and were blunt-cloned into the BamHI site of the expression vector pKG5 (2).

Transfection by Electroporation. Cells were transfected by electroporation as described previously (2). Cells containing the *neo* gene were selected with G418 at a concentration of 1 mg/ml. Subsequently, cells expressing high levels of CD16 were selected by FACS (Becton Dickinson & Co., Mountain View, CA).

Flow Cytometric Analysis. Cells, 3 × 10⁶ per sample, were incubated with 50 μl of antibody (tissue culture supernatant) for 1 h on ice. Cells were washed twice at 4°C in 1 ml of PBS containing 0.25% BSA (PBS/BSA) and 10 mM NaN₃, and were subsequently incubated in 50 μl of FITC-conjugated rabbit anti-mouse IgG (RAM) (15 μg/ml) (29). Cells were resuspended in 0.4 ml PBS/BSA and analyzed on a FACScan® (Becton Dickinson & Co.).

Saturation Binding Assay. Cells were incubated with saturating amounts of mAbs as for flow cytometric analysis. For CD2, the primary mAb used was OKT11 (IgG1) while the mAb 3G8 (IgG1) was used for CD16. After three washes in 1 ml of PBS/BSA at 4°C, cells were incubated with 25 μl of ¹²⁵I-RAM IgG F(ab')₂ at 30 μg/ml (500,000 cpm). After 1 h on ice, cells were washed three times as above and bound radioactivity was counted. Site numbers were determined by comparison of radioactivity bound by the W3/25 mAb to rat thymocytes (15,000 sites per cell) (30).

Glycosyl-Phosphatidyl Inositol-specific Phospholipase C (PI-PLC) Treatment of Jurkat Cells. CD16 (GPI)⁺ and CD16 (T/M)⁺ Jurkat cells (10⁷ each) were incubated with 1.8 × 10⁻³ U of Bacillus thuringiensis PI-PLC (Peninsula Laboratories, Merseyside, UK) in 200 μl of PBS/BSA 0.5% at 37°C for 1 h. The cells were washed twice with PBS/BSA 0.5%, split into three aliquots of 3.3 × 10⁶ cells, and labeled for flow cytometric analysis as described above.

Measurement of [Ca²⁺]_i. [Ca²⁺]_i was determined as described previously (31).

Preparation of F(ab')₂ Fragments. OX-34 F(ab')₂ was prepared by pepsin digestion of IgG in Na acetate buffer (pH 4.0) for 10 h at 37°C (32). After dialysis in a Tris saline buffer (pH 7.6), the F(ab')₂ and Fab fragments were separated by gel filtration on a Sephadex G-200 column (Pharmacia Ltd., Bucks, UK). The fractions containing the F(ab')₂ were pooled and passed over a protein A (Pharmacia Ltd.) column three times. This reduced the contamination of IgG to <0.001% (data not shown).

F(ab')₂ RAM was first prepared by pepsin digestion and gel filtration, and samples judged to be free of IgG as assessed by SDS-PAGE were pooled (29). Analysis by inhibition of protein A binding showed the presence of 1% IgG (Fig. 1). The F(ab')₂ RAM was then further purified by passage over a protein A column three times. This reduced the contamination to <0.0001% (Fig. 1).

Inhibition Binding Assay. F(ab')₂ RAM was assayed for the presence of IgG by inhibition of an indirect radioactive binding assay

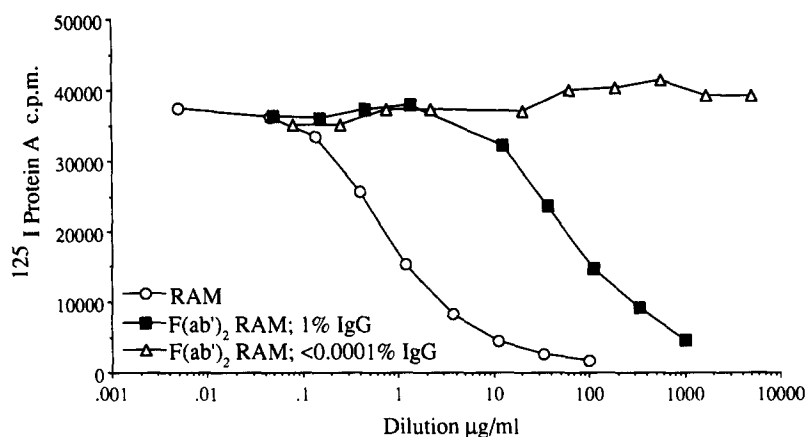


Figure 1. Inhibition binding assay to quantitate the amount of IgG in the preparations of F(ab')₂ RAM before and after passage over a protein A column. Experimental details are given in Materials and Methods.

using [¹²⁵I]protein A (Amersham International, Amersham, UK). For targets in the assay, rabbit IgG (RAM) was coated onto wells of a flexible assay plate (3911; Falcon Labware, Oxnard, CA) by incubation of 50 µl at 50 µg/ml for 30 min at 20°C. The unbound RAM was then removed by washing with Dulbecco's A + B PBS solution (DAB) (Oxoid Ltd., Basingstoke, UK). The plate was blocked with DAB/BSA 0.5% and then washed again using DAB. A range of serial dilutions were made of RAM and F(ab')₂ RAM and 50 µl aliquots were added to 120,000 cpm of ¹²⁵I-protein A in 25 µl and incubated at 4°C for 1 h. 50 µl aliquots were then transferred to the plate coated with RAM and incubated at 4°C for 1 h, washed, and dried at 37°C. The bound radioactivity was counted on a gamma counter.

Results

Expression of Two Molecular Forms of CD16 on TCR⁻ Jurkat Cells. cDNA encoding the glycosyl phosphatidylinositol (GPI)-linked and T/M forms of CD16 were cloned into the expression vector pKG5 and transfected into the TCR⁻ Jurkat cell line J.RT3-T3.5. Flow cytometry showed that the two molecular forms of CD16 were similarly expressed on the transfected Jurkat cells. Control and transfected cells were homogeneously positive for CD2, whereas CD3 was not detectable (Fig. 2). The expression of the two forms of CD16 and of CD2 was quantitated by a saturation binding assay. CD16 (T/M) and CD16 (GPI) were expressed at 2.4×10^4

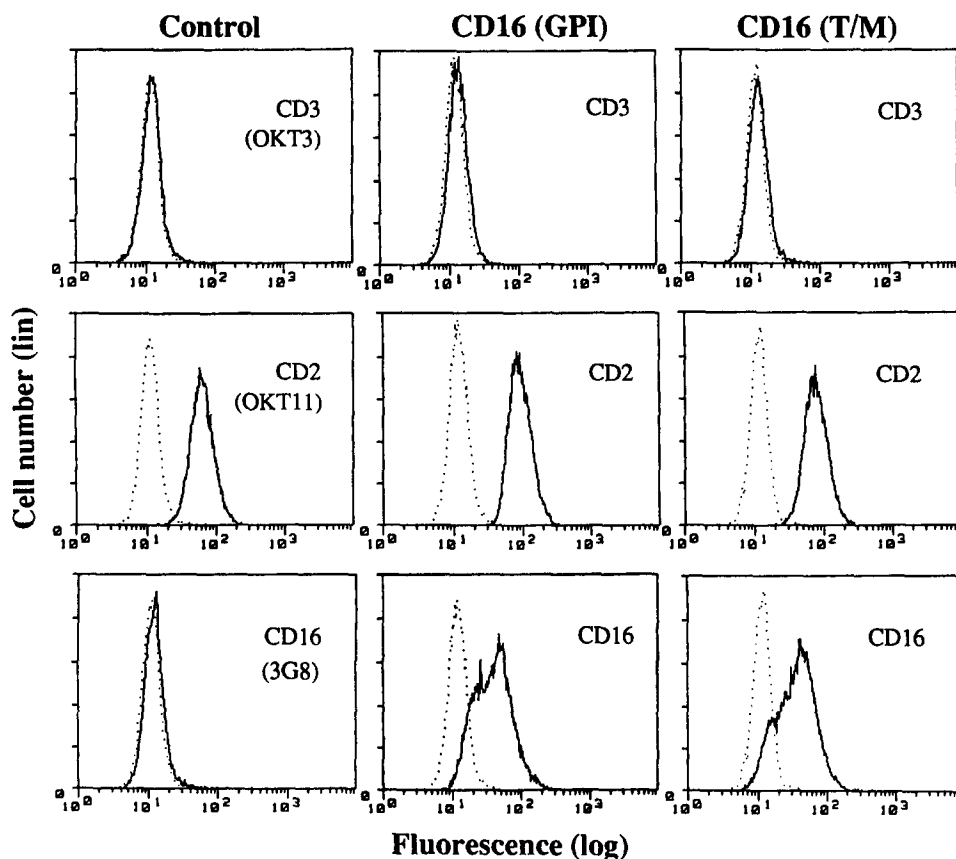


Figure 2. Flow cytometric analysis of TCR⁻ Jurkat cells expressing the (T/M) or GPI-linked form of CD16. Untransfected TCR⁻ Jurkat cells were used as controls. The dotted lines show labeling with an isotype-matched negative control mAb F23.1 which was used in the case of OKT3, and OX-21 in the case of OKT11 and 3G8.

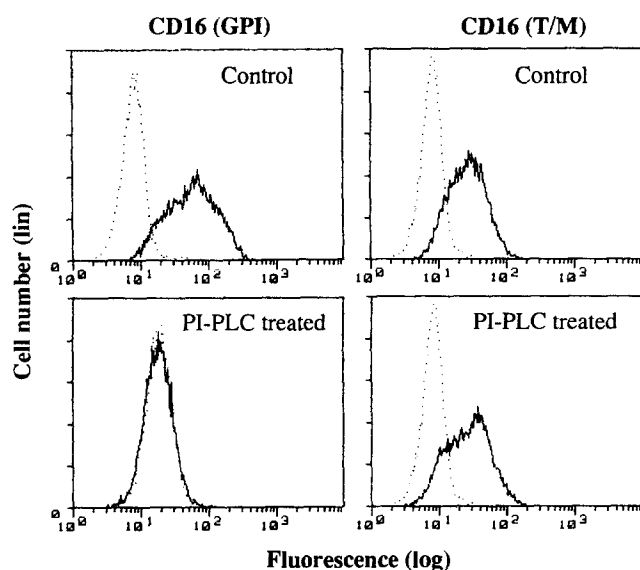


Figure 3. PI-PLC treatment of TCR⁻ Jurkat cells expressing the (T/M) or GPI-linked form of CD16. Cells were incubated in PBS/BSA 0.5% with or without PI-PLC. The dotted lines show labeling with an isotype matched negative control mAb, OX-21. The CD16 antigen was labeled with the mAb 3G8.

molecules per cell and 3.4×10^4 molecules per cell, respectively, while CD2 was expressed at 4×10^4 molecules per cell in all three lines. CD16 was cleaved from the surface of the CD16 (GPI) line by PI-PLC treatment. This enzyme did not cleave CD16 from the surface of the CD16 (T/M)⁺ Jurkat cell line (Fig. 3).

Ca²⁺ Signals Triggered via CD2 and CD16 in Transfected Jurkat Cell Lines. Increases in [Ca²⁺]_i were determined in Jurkat cells, untransfected or expressing the two forms of CD16, after stimulation with antibodies directed against CD3, CD2, and CD16. The primary antibodies were crosslinked by F(ab')₂ fragments of RAM containing either 1% IgG or <0.0001% IgG. Representative profiles of Ca²⁺ signals are shown in Fig. 4, and quantitative data are shown in Table 1. As could be expected from the inability to detect surface expression of CD3 as measured by flow cytometry, only small increases in [Ca²⁺]_i were seen when CD3 was crosslinked using F(ab')₂ fragments of anti-CD3 mAbs.

Signaling via CD16 required the addition of a crosslinker and was only seen in the cells expressing CD16 (T/M), not in the cells expressing CD16 (GPI). The signal transduced via CD16 in the CD16 (T/M) population was of the same

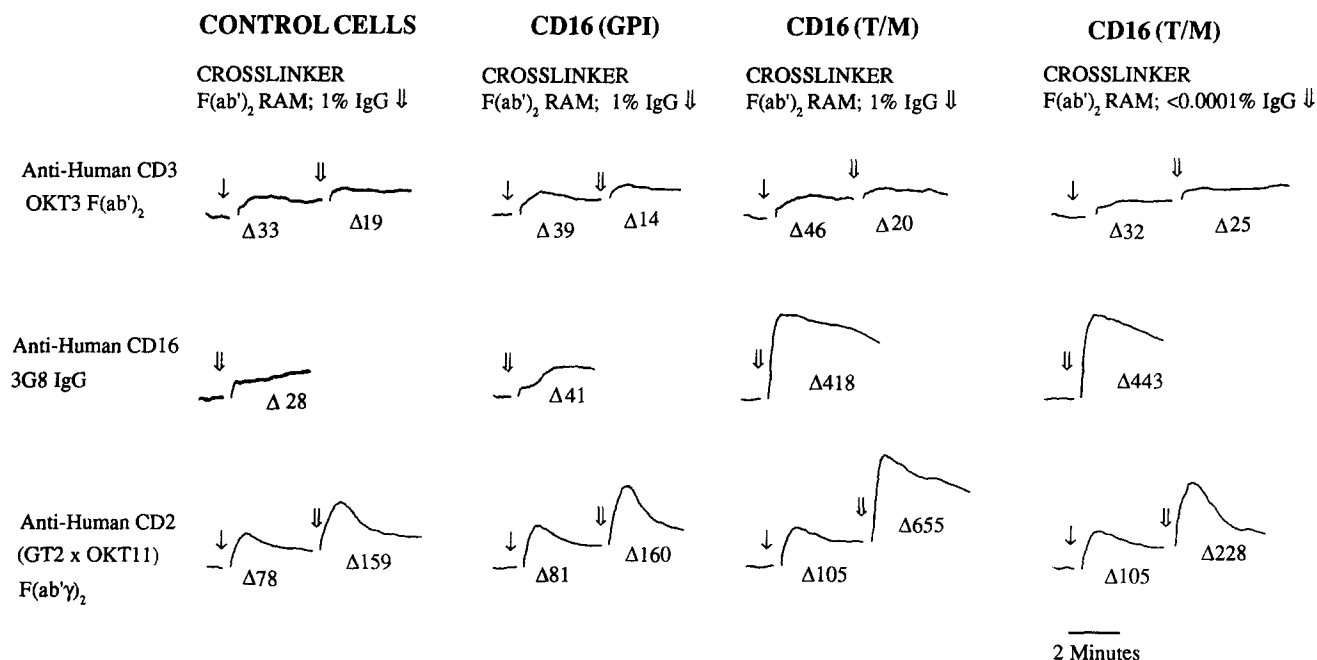


Figure 4. [Ca²⁺]_i responses of TCR⁻ Jurkat cells expressing the T/M form of CD16 using as crosslinker F(ab')₂ RAM with 1% IgG and F(ab')₂ RAM with <0.0001% IgG. Untransfected Jurkat cells or TCR⁻ Jurkat cells expressing the GPI-linked form of CD16 were used as controls and were crosslinked with F(ab')₂ RAM containing 1% IgG. The cells were loaded with fura-2 AM, and at the time of points indicated (↓), anti-human CD2 mAb (bispecific [GT2 × OKT11] F[ab'γ]₂) (2.5 μg/ml final concentration) or anti-human CD3 mAb (OKT3 F[ab']₂) (0.5 μg/ml final concentration) were added. In addition cells were pre-incubated for 1 h on ice in 50 μl of 50 μg/ml anti-human CD16 mAb (3G8) and then diluted to 2 ml before addition of the crosslinking mAb. The addition of the crosslinking mAb (20 μg/ml final concentration) is indicated (↑). Values following the Δ indicate increases in [Ca²⁺]_i in nanomolar above the base lines and the time is indicated by the horizontal bar. Baseline values were 100 ± 26 nM. The [Ca²⁺]_i increases are on a nonlinear scale. Gaps in the traces are due to additions and stirring of the suspensions. All results are representative of three experiments and data in vertical groups were collected at one time.

Table 1. Increases in $[Ca^{2+}]_i$ Induced by Various Ligands and Crosslinkers in TCR⁻ Jurkat Cells

Triggering agent	Control cells		CD16 (GPI)		CD16 (T/M)		
	No crosslinker	F(ab') ₂ RAM; 1% IgG	No crosslinker	F(ab') ₂ RAM; 1% IgG	No crosslinker	F(ab') ₂ RAM; 1% IgG	F(ab') ₂ RAM; <0.0001% IgG
<i>nM</i>							
Anti-human CD3							
OKT3 F(ab') ₂	31 ± 1	16 ± 2	30 ± 5	29 ± 8	35 ± 4	23 ± 3	12 ± 7
Anti-human CD2							
(GT2 × OKT11)F(ab'γ) ₂	76 ± 3	169 ± 17	76 ± 5	172 ± 14	91 ± 4	627 ± 30	203 ± 30
Anti-human CD16							
3G8 IgG	-	23 ± 5	-	46 ± 6	-	447 ± 16	400 ± 25

Cell populations and ligands are as for Fig. 4. The final concentration of OKT3 F(ab')₂ was 0.5 μg/ml, and that of (GT2 × OKT11)F(ab'γ)₂ was 2.5 μg/ml. Cells were pre-incubated on ice for 1 h in 50 μl of 50 μg/ml 3G8. The final concentration of the crosslinking mAb was 20 μg/ml. Values are differences in $[Ca^{2+}]_i$ before and after the addition of the ligands, and each represents the mean ± SEM of three experiments. Baseline values were 100 ± 26 nM. In experiments where a gradual rise in baseline was observed, corrections were made for leakage of fura-2.

order of magnitude with both preparations of F(ab')₂ RAM crosslinker.

T cell activation via human CD2 requires crosslinking of two CD2 epitopes. To achieve effective crosslinking, bispecific (GT2 × OKT11) F(ab'γ)₂ was prepared. This bispecific anti-CD2 mAb generated signals of a similar magnitude in the control Jurkat cells and in the Jurkat cells expressing CD16 (GPI), both before and after further crosslinking with F(ab')₂ RAM containing 1% IgG. In Jurkat cells expressing the T/M form of CD16, there was a similar rise in $[Ca^{2+}]_i$ after the addition of the primary bispecific anti-CD2 mAb. After additional crosslinking with F(ab')₂ RAM containing 1% IgG, however, there was a prompt large rise in $[Ca^{2+}]_i$. After removal of the 1% IgG from the F(ab')₂ RAM, the enhanced signal above that seen on untransfected cells or cells transfected with CD16 (GPI) was no longer observed.

The effect of the small amount of IgG on $[Ca^{2+}]_i$ levels was reflected not only in the magnitude of the Ca^{2+} signals, but also in their kinetics. In the untransfected cells as well as in the cells expressing CD16 (GPI) and CD16 (T/M), the bispecific anti-CD2 mAb induced a sharp rise in $[Ca^{2+}]_i$ followed by a return to levels <50 nM above the baseline within 3 min of adding the primary mAb. Within 3 min of adding

the crosslinker, $[Ca^{2+}]_i$ levels were <40 nM above those attained before crosslinking in the untransfected cells and the cells expressing CD16 (GPI). In cells expressing CD16 (T/M), however, crosslinking of anti-CD2 mAb with F(ab')₂ RAM containing 1% IgG induced a sustained increase in $[Ca^{2+}]_i$. 3 min after adding the crosslinker, the $[Ca^{2+}]_i$ levels were ~200 nM above those observed immediately before the addition of the crosslinker. Similar profiles were obtained using a bispecific (GT2 × OKT11) F(ab')₃ comprising two OKT11 Fab' arms and one GT2 Fab' arm (data not shown).

Ca²⁺ Signals Generated via CD2 in the Rat NK Cell Line. The results in Jurkat cells suggested that the role of the CD2 antigen in signal transduction on NK cells should be re-examined. The previously described rat NK cell line RNK-16 (14) was studied for this purpose. The cells express high levels of CD2, are positive for the NK cell marker NKR-P1 (26), and are negative for CD3 (Fig. 5).

Representative profiles of Ca^{2+} signals generated via CD2 using the anti-CD2 mAb OX-34 in the form of IgG and F(ab')₂ are shown in Fig. 6, and quantitative data are given in Table 2. When OX-34 IgG was used, the largest rise in $[Ca^{2+}]_i$ was obtained using IgG RAM as crosslinker. The Ca^{2+} signal was reduced when OX-34 IgG was crosslinked

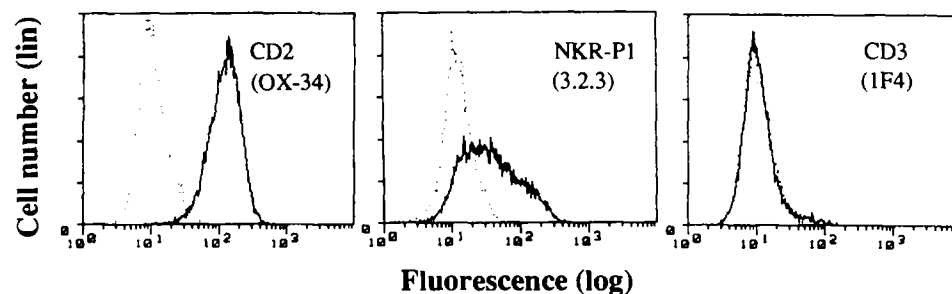


Figure 5. Flow cytometric analysis of a rat NK cell line RNK-16. The CD2, NKR-P1, and CD3 antigens were labeled with the OX-34, 3.2.3, and 1F4 mAbs, respectively. The dotted lines show labeling with isotype-matched negative control antibodies F23.1 (IgG2a) in the case of OX-34, OX-21 (IgG1) in the case of 3.2.3, and W6/1 (IgM) in the case of 1F4.

NK CELL LINE: RNK-16

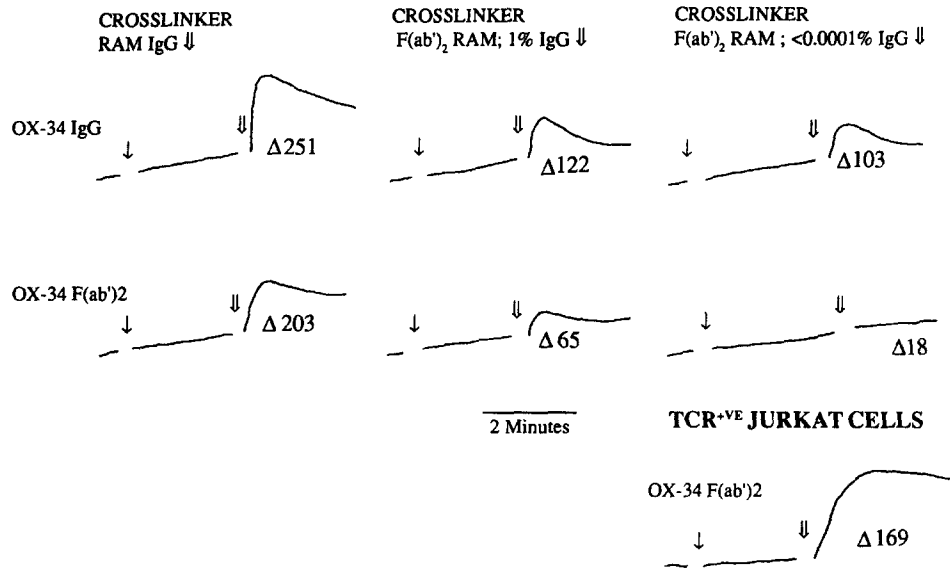


Figure 6. $[Ca^{2+}]_i$ responses of a rat NK cell line RNK-16 and of TCR⁺ Jurkat cells expressing rat CD2. The OX-34 mAb was used as whole IgG or as OX-34 F(ab')₂ containing <0.001% IgG (2 μ g/ml final concentration) and crosslinked with RAM, F(ab')₂ RAM containing 1% IgG or F(ab')₂ RAM containing <0.0001% IgG (20 μ g/ml final concentration). mAbs (\downarrow) and crosslinkers (\Downarrow) were added to the cells at the time points indicated. Values following the Δ indicate increases in $[Ca^{2+}]_i$ in nanomolar above the base lines and the time is indicated by the horizontal bar. Baseline values were 136 ± 14 nM. The $[Ca^{2+}]_i$ increases are on a nonlinear scale. Gaps in the traces are due to additions and stirring of the suspensions.

with F(ab')₂ RAM containing 1% IgG, and a similar sized signal was seen when pure F(ab')₂ RAM crosslinker was used. Crosslinking of OX-34 F(ab')₂, which contained <0.001% of IgG (data not shown) with IgG RAM, gave a large signal that was diminished when the RAM was replaced by F(ab')₂ RAM containing 1% IgG as crosslinker. No signal was transduced when the OX-34 F(ab')₂ was cross-linked using pure F(ab')₂ RAM. A similar titration effect was seen in a second rat NK cell line, A181, which was maintained by passage in Fisher rats (L.L. Spruyt and M.J. Glennie, unpublished data). In this line, the crosslinking of OX-34 F(ab')₂ (<0.001% IgG) with highly purified F(ab')₂ RAM (<0.0001% IgG) also failed to transduce a signal. The functional effectiveness of the OX-34 F(ab')₂ was tested on a TCR⁺ Jurkat cell line expressing rat CD2 (2). Crosslinking of rat CD2 on these cells, using the OX-34 F(ab')₂ followed by pure F(ab')₂ RAM, induced a rapid and sustained increase in $[Ca^{2+}]_i$ (Fig. 6).

Discussion

There is a substantial amount of evidence that the TCR/CD3 complex is required for efficient signaling via CD2 (1, 2). The key points are as follows: (a) downmodulation of CD3 prevents stimulation via CD2 (9); (b) CD2 expressed on an insect gut epithelial line (7) or a murine L fibroblast line (8) does not function as a target for signal transduction; (c) cell lines that lack the TCR/CD3 complex or that are defective in their TCR/CD3 complex show reduced signaling via CD2.

There is some ambiguity in the data with respect to the Jurkat T cell leukemic lines. The TCR/CD3⁺ CD2⁺ line J.Cam1, which has a defective signaling pathway through the TCR/CD3 complex, is not responsive to CD2 ligands. Correction of the signaling defect restores the response (33), demonstrating the necessity for a functional TCR/CD3 complex. Other studies (33–35) also showed that CD2 does not function as a target for activation in TCR/CD3[−] Jurkat

Table 2. Increases in $[Ca^{2+}]_i$ Induced in the Rat NK Cell Line RNK-16

OX-34 IgG			OX-34 F(ab') ₂		
RAM	F(ab') ₂ RAM; 1% IgG	F(ab') ₂ RAM; <0.0001% IgG	RAM	F(ab') ₂ RAM; 1% IgG	F(ab') ₂ RAM; <0.0001% IgG
nM					
232 \pm 10	108 \pm 10	83 \pm 11	180 \pm 12	48 \pm 10	10 \pm 5

Cell populations and ligands are as for Fig. 6. The final concentration of OX-34 IgG and OX-34F(ab')₂ was 2 μ g/ml, and that of the crosslinking mAb 20 μ g/ml. Values are differences in $[Ca^{2+}]_i$ before and after the addition of the ligands, and each represents the mean \pm SEM of three experiments. Baseline values were 136 ± 14 nM. In experiments where a gradual rise in baseline was observed, corrections were made for leakage of fura-2.

lines. In Jurkat cells lacking the TCR β chain, signaling did not occur and transfection with β chain cDNA restored the potential to be activated via crosslinking of CD2 (33, 35). However, in other studies, activation via CD2 has been reported to occur in TCR/CD3⁻ Jurkat cell lines, and this was correlated with CD2 expression at high levels by Ohno et al. (34). In another case (36), activation via CD2 on TCR/CD3⁻ Jurkat cell lines has been attributed to the presence of TCR/CD3 expression in amounts below the limits of detection by flow cytometry (35). Overall, the results argue that efficient activation via CD2 requires the presence of the TCR/CD3 complex.

The present experiments were done with the TCR/CD3⁻ Jurkat cell line J.RT3-T3.5, which is mutant for TCR β chain expression and lacks detectable CD3 expression at the cell surface. In previous studies, rat CD2 transfected into these cells did not provide a target for activation in comparison with effective signal transduction via rat CD2 on TCR/CD3⁺ Jurkat cells (2). In contrast to the rat CD2 results, crosslinking of human CD2 on the TCR⁻ cells in the current work did give a Ca^{2+} signal. The crosslinking reagent was a F(ab')_2 heterodimer reacting with two different epitopes of CD2 and this was further crosslinked with F(ab')_2 RAM. However, a much larger signal was seen when the TCR⁻ cells were transfected with CD16 (T/M) and crosslinked with anti-CD16 antibody. Also on the CD16 (T/M)⁺ cells, a large signal was seen with crosslinking via CD2, but only when the crosslinking F(ab')_2 RAM contained some IgG. Without this the expression of CD16 (T/M) did not potentiate larger signals via the CD2 molecule and transfection with CD16 (GPI) did not potentiate signals under any circumstances. There are numerous studies showing that expression of CD16 (T/M) at the cell surface requires association with ζ or γ chains but not other chains of the TCR/CD3 complex (13). Thus, we conclude that the expression of ζ chains in a complex lacking the rest of the TCR/CD3 molecules does not potentiate signaling via CD2.

The results in Jurkat cells lead us to re-examine signal trans-

duction via CD2 on NK cells using the OX-34 mAb in the form of F(ab')_2 that contained <0.001% IgG. In this NK cell line and also in line A181, which has recently arisen in a Fisher rat (L.L. Spruyt and M.J. Glennie, unpublished data), there was no rise in $[\text{Ca}^{2+}]_i$ after crosslinking of CD2 in the absence of involvement of the Fc receptor CD16 (T/M).

The present studies in TCR⁻ Jurkat cells and NK cells are in accord with recent data in which a chimeric protein, made by linking the extracellular and transmembrane domains of CD8 to the cytoplasmic domain of ζ , was transfected into the TCR/CD3⁻ J.RT3-T3.5 Jurkat cell line and was expressed at the cell surface as a homodimer. These Jurkat cells, expressing the chimeric CD8⁻ ζ mutants in the absence of a TCR/CD3 complex, were unresponsive to combinations of stimulating anti-CD2 mAbs as measured by calcium fluorimetry (37).

An Fc γ receptor ectodomain-independent triggering mechanism has recently been proposed in which ζ/γ dimers may confer a TCR-independent signal transduction pathway upon CD2 in TCR/CD3⁻ T cells or NK cells (38). In this system, stimulation of human CD2, expressed on the surface of a murine mast cell line, leads to a rise in $[\text{Ca}^{2+}]_i$, IL-6 production, and histamine release. These results were obtained using the anti-CD2 mAbs both in the form of whole IgG and as F(ab')_2 fragments that were >95% pure on SDS-PAGE. Given the pronounced effect of trace amounts (1%) of IgG in our F(ab')_2 RAM preparation, it is possible that effects demonstrated in the murine mast cell line were due to co-stimulation of CD16 (T/M) and CD2.

Taken together, the results in NK cells indicate that, despite the expression of functional ζ/γ dimers, CD2 appears to require additional elements such as other CD3 chains to couple it to the cellular signal transduction pathways. The primary function of CD2 may be to act as an adhesion molecule synergizing with the TCR/CD3 signal transduction complex in T cells when antigen is recognized in the context of MHC or synergizing with the Fc receptor complex Fc γ RIIIA when immune complexes are bound by NK cells.

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Address correspondence to Alan F. Williams, MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK.

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