Antigen endocytosis and presentation mediated by human membrane IgG1 in the absence of the Igα/Igβ dimer

Andrew M.Knight, John M.Lucocq1, Alan R.Prescott, Sreenivasan Ponnambalam and Colin Watts

Departments of Biochemistry and 1Anatomy and Physiology, Medical Sciences Institute, University of Dundee, Dundee, DD1 4HN, UK

Membrane immunoglobulin (mIg) M and D heavy chains possess minimal (KVK) cytoplasmic tails and associate with the Igζ/Igβ (CD79) dimer to achieve surface expression and antigen presentation function. In contrast, the cytoplasmic tail of mIgG is extended by 25 residues (ζct). We have tested the possibility that mIgG can perform antigen capture and presentation functions independently of the Igα/β dimer. We show that CD4/ζct chimeras are efficiently endocytosed partially dependent on a tyrosine residue in ζct. In addition, human mIgG was expressed on the surface of Igα/β-negative non-lymphoid cells and mediated antigen capture and endocytosis. Antigen-specific human mIgG targeted antigen to MHC-type vesicles in the Igα/β-negative melanoma Mel JuSo and augmented antigen presentation 1000-fold, identical to the augmentation seen in Igα/β-positive B-cells expressing the same transfectected mIgG. Thus, unlike mIgM, mIgG has autonomous antigen capture and presentation capacity, which may have evolved to reduce or eliminate the BCR’s dependence on additional accessory molecules.

Keywords: antigen presentation/class II MHC/endocytosis/Igζ/Igβ/mIgG/

Introduction

The B-cell antigen receptor (BCR) is a membrane-anchored immunoglobulin (mIg) (for review, see Reth, 1992; Cambier et al., 1994) whose expression on the cell surface is essential for normal B-cell maturation beyond the pre-B-cell stage (Kitamura et al., 1991). The BCR is composed of heavy and light immunoglobulin chains and a non-covalently associated disulfide-linked dimer of Igζ and Igβ proteins (Hombach et al., 1988). Several features of BCR function depend on co-expression of this dimer. In its absence, mIgM cannot be expressed on the cell surface (Hombach et al., 1990; Williams et al., 1990; Venkitaraman et al., 1991; Matsuuchi et al., 1992), BCR signalling capacity is lost (Grupp et al., 1993; Sanchez et al., 1993; Michnoff et al., 1994; Williams et al., 1994) and consequently, normal B-cell maturation is disrupted (Papavasiliou et al., 1995a,b; Gong and Nussenzweig, 1996; Torres et al., 1996).

B-cells expressing antigen-specific BCRs can present antigens to MHC class II-restricted T-cells 10^3- to 10^4-fold more efficiently than non-specific B-cells (Rock et al., 1984; Lanzavecchia, 1985). This is due to specific antigen binding, endocytosis and delivery for processing to the MHC class II-rich endosomal compartments (MIIC/CtIV) found in these cells (Peters et al., 1991; Amigorena et al., 1994; reviewed in Watts, 1997). The Igζ/Igβ dimer has also been demonstrated to play an important role in BCR antigen presentation function. Since mIgM (μ) and mIgD (δ) have transmembrane heavy chains with only three putative cytoplasmic residues (~KVK), the Igζ/Igβ dimer is thought to act as an adaptor permitting the external antigen binding site to be internalized and access the endocytic pathway. Indeed, the cytoplasmic domain of Igβ was able to confer endocytosis and antigen presentation capacity when directly fused to mIg or Fc receptors (Patel and Neuberger, 1993; Bonnerot et al., 1995), although in one study only the tail of Igα appeared able to target FcR-bound antigen to newly synthesized class II MHC molecules (Bonnerot et al., 1995).

Following antigen stimulation and isotype switching, B-cells continue to express membrane-anchored immunoglobulins. However, the isotype-switched immunoglobulin classes IgG (γ), IgA (α) and IgE (ε) possess extended cytoplasmic tails encoded by the M2 exon (Bensman and Lefranc, 1990; Kinoshita et al., 1991; Sun et al., 1991, respectively). In the case of IgM, the KVK sequence is extended by 25 residues to form a cytoplasmic tail (ζct) which is highly conserved among γ sub-types and between species (Bensman and Lefranc, 1990; Kinoshita et al., 1991). No function for the cytosolic domains of class-switched membrane Igs has been described. We have examined the possibility that the extended heavy chain of mIgM may permit BCR cell surface expression and antigen presentation function, independent of the Igζ/Igβ dimer. We demonstrate for the first time that the cytoplasmic tail of a human mIgG contains an endocytosis motif. Further, we show that the mIgG BCR can be expressed on the surface of cells lacking the Igζ/Igβ dimer and that this minimal form of the BCR can provide the full 10^3- to 10^4-fold enhancement of antigen presentation characteristic of antigen-specific B-cells.

Results

The cytoplasmic tail of membrane IgG1 heavy chain can mediate endocytosis

To initiate studies on the mIgG form of the BCR we used RT–PCR to clone the heavy and light chain genes (see Materials and methods) from the Epstein–Barr Virus (EBV)-transformed human B-cell clone 11.3 which expresses membrane IgG1 specific for tetanus toxin (Lanzavecchia, 1985). The predicted γ heavy chain was 521 residues in length and, compared with membrane μ chains, was extended as expected by 25 C-terminal residues (ζct; Figure 1A). In clone 11.3 cells we found an
asparagine residue at position 512 instead of the aspartic acid reported for other human membrane gamma chains (Bensmana and Lefranc, 1990; Kinoshita et al., 1991).

We first tested the capacity of the cytoplasmic tail of mlgM (γt) to mediate endocytosis by creating chimeras in which γt was transplanted onto the extracellular and transmembrane domains of CD4 (CD4/γt; Figure 1A). We compared the endocytosis kinetics of this construct with that of two other CD4 derivatives, CD4stop and CD4ac (Figure 1A) which show basal endocytosis rates (Figure 3A, panel e) and are endocytosed faster than CD4stop or CD4ac and eventually equilibrated with a similar-sized intracellular pool as CD4/γt (Figure 1B). These data suggest that the γt domain contains at least two endocytosis signals, one of which is based around Y513.

Moreover, since the rate of ligand clearance from the cell surface and the steady-state pool size observed with the CD4/γt chimera is comparable with previously observed for specific antigen uptake via mlgG on human B-cell lines (Watts and Davidson 1988; Davidson and Watts 1989), this raised the possibility that γt might be involved in antigen endocytosis via mlgG BCRs.

Surface expression and endocytosis of mlgG in the absence of both Igα and Igβ

Several studies demonstrate that mlgM (μ) requires co-expression of the Igα/Igβ dimer in order to be exported from the endoplasmic reticulum (Hombach et al., 1988; Matsuuchi et al., 1992). However, the murine γb2 and γa2a isotypes, together with associated light chain, were able to reach the cell surface of J558L plasmacytoma cells which lack the Igα sub-unit (Venkitaraman et al., 1991; Weiser et al., 1994). We therefore tested whether the human γ1 isotype can reach the cell surface in the absence of both Igα and Igβ by expressing the light chain (κ) gene along with either wild-type or mutant γ chains in HeLa cells which are Igα/Igβ-negative (Verschuren et al., 1993). When expressed alone, the γ heavy chain failed to reach the cell surface and biosynthetically labelled material remained endoglycosidase H-sensitive (Figure 2). In contrast, co-transfection with light chains resulted in association with the γ heavy chain, acquisition of endoglycosidase H-resistance and transport to the cell surface (Figure 2). Indirect immunofluorescence microscopy confirmed that the heavy/light chain complex had reached the cell surface (Figure 3A, panel b) whereas the heavy chain expressed alone did not (Figure 3A, panel a) and instead was retained in the endoplasmic reticulum (Figure 3A, panel c'). Thus, unlike mlgM, mlgG can be exported to the cell surface of non-lymphoid cells in the absence of both Igα and Igβ.

Interestingly, export of γκ complexes from the endoplasmic reticulum appeared to be partially dependent on γt since deletion of the 25 residue γ-specific tail (H(KVK), Roth, 1994), while not affecting the level of surface expression (Figure 3A, panel d), caused significant accumulation of mlgG in the endoplasmic reticulum (Figure 3A, panels h and h'). Mutagenesis of the single tyrosine (Y513A) within γt could still be endocytosed faster than CD4stop or CD4ac and eventually equilibrated with a similar-sized intracellular pool as CD4/γt (Figure 1B). These data suggest that the γt domain contains at least two endocytosis signals, one of which is based around Y513.
A.M.Knight et al.

Fig. 2. Cell surface expression of mlgG in the absence of the Igα/Igβ dimer. Stable HeLa transfectants were generated expressing either Igγ heavy chain cDNA (left-hand panels) or Igγ heavy and κ light chain cDNAs (right-hand panels). Top panels show flow cytometric analysis (10^5 cells) stained with biotinylated goat anti-human IgG followed with streptavidin FITC (IGG) or with streptavidin FITC only (2ND ONLY). Bottom panels show immunoprecipitated (arrows) heavy (H) (left panel) or heavy and light (L) chains (right panel) from 35S-labelled transfectants treated (+) or mock-treated (−) with endoglycosidase H.

These vesicles were also evident in the H(Y513A) mutant (Figure 3A, panels g and g'), but they were much less prominent in cells transfected with the H(KVK) γ chain where most intracellular mlgG appeared to be confined to the endoplasmic reticulum (Figure 3A, panels h and h'). To establish whether these vesicles were endocytic in origin we incubated transfected HeLa cells with biotinylated goat anti-human IgG followed with streptavidin FITC (IGG) or with streptavidin FITC only (2ND ONLY). These experiments confirmed that the MIIC-like structures previously observed in Ramos (Figure 4A). Pilot experiments also showed that Mel JuSo cells could process and present the tetanus toxin epitope 1273–1284 to the DRw52a/c-restricted T-cell clone KT4 (Demotz et al., 1989) following antigen pulsing (Figure 4B). As expected, both Ramos (Figure 5B) and A20 (not shown) expressed the intact or the tailless mlgG [H(KVK)] on the cell surface (Figure 5A). As previously seen in HeLa cells, Mel JuSo transfectants also expressed the intact or the tailless mlgG [H(KVK)] on the cell surface in the absence of Igα/Igβ (Figure 5A). In addition, immunoelectron microscopy of the Mel JuSo transfectants revealed that the MIIC-like structures previously observed in this cell line (Pieters et al., 1991) also contained the transfected mlgG (Figure 5B), indicating its successful targeting to this specialized endosome population in the absence of Igα/Igβ expression.

To test antigen presentation capacity, transfectants were cultured together with graded doses of antigen in the presence of tetanus toxin-specific T-cell clones. Introduction of tetanus toxin-specific mlgG into the Ramos cell line allowed antigen presentation to the T-cell clone D1c at concentrations 10^3 to 10^4-fold lower than those required for untransfected Ramos cells (Figure 6A). This is consistent with earlier studies which demonstrated a very similar enhancement of antigen presentation in antigen-specific versus non-specific B-cells (Rock et al., 1984; Lanzavecchia, 1985). We next tested the relative efficiency of antigen presentation in control Mel JuSo cells versus Mel JuSo clones expressing the same antigen-specific heavy and light chains. Remarkably, the same dramatic augmentation of antigen presentation was observed in Mel JuSo cells expressing wild-type mlgG as was observed in Ramos (Figure 6B and C). Thus, the mlgG BCR can enhance antigen presentation to the same extent in the presence or absence of the Igα/Igβ dimer. To assess the contribution that γct might make to antigen presentation via the mlgG BCR we also tested antigen presentation in Ramos B cells and Mel JuSo cells expressing a heavy chain lacking the cytoplasmic tail [H(KVK)]. With this truncated form of the BCR, Ramos transfectants presented antigen at least as well as Ramos transfected with full-length γ heavy chains (Figure 6A). Similar results were obtained in A20 murine B-cells transfected with the same mlgG BCR constructs (not shown). In these experiments we utilized a tetanus toxin/ovalbumin conjugate and the ovalbumin-specific T-cell hybridoma 3DO.548 to assay antigen presentation (data not shown).

We next assessed whether mlgG antigen presentation function requires co-expression of Igα/Igβ. We therefore introduced mlgG into both Igα/Igβ-positive B-cells (Ramos; human and A20; mouse) and into the HLA-DR- and DM-positive melanoma line Mel JuSo. This non-lymphoid cell line contains class II MHC-positive endosomes (Pieters et al., 1991; Sanderson et al., 1994; Tulp et al., 1994) similar to the MIIC vesicles identified in B-lymphocytes and dendritic cells (Peters et al., 1991; Nijman et al., 1995). Importantly, the Mel JuSo transfectants were negative for the Igα/Igβ complex as assessed by staining with the CD79b (Igβ)-specific monoclonal antibody CB3-1 (Nakamura et al., 1992) which detected abundant expression on the Burkitt lymphoma cell line Ramos (Figure 4A). Pilot experiments also showed that Mel JuSo cells could process and present the tetanus toxin epitope 1273–1284 to the DRw52a/c-restricted T-cell clone KT4 (Demotz et al., 1989) following antigen pulsing (Figure 4B). As expected, both Ramos (Figure 5B) and A20 (not shown) expressed the intact or the tailless mlgG [H(KVK)] on the cell surface (Figure 5A). As previously seen in HeLa cells, Mel JuSo transfectants also expressed the intact or the tailless mlgG [H(KVK)] on the cell surface in the absence of Igα/Igβ (Figure 5A). In addition, immunoelectron microscopy of the Mel JuSo transfectants revealed that the MIIC-like structures previously observed in this cell line (Pieters et al., 1991) also contained the transfected mlgG (Figure 5B), indicating its successful targeting to this specialized endosome population in the absence of Igα/Igβ expression.

We next tested the relative efficiency of antigen presentation in control Mel JuSo cells versus Mel JuSo clones expressing the same antigen-specific heavy and light chains. Remarkably, the same dramatic augmentation of antigen presentation was observed in Mel JuSo cells expressing wild-type mlgG as was observed in Ramos (Figure 6B and C). Thus, the mlgG BCR can enhance antigen presentation to the same extent in the presence or absence of the Igα/Igβ dimer. To assess the contribution that γct might make to antigen presentation via the mlgG BCR we also tested antigen presentation in Ramos B cells and Mel JuSo cells expressing a heavy chain lacking the cytoplasmic tail [H(KVK)]. With this truncated form of the BCR, Ramos transfectants presented antigen at least as well as Ramos transfected with full-length γ heavy chains (Figure 6A). Similar results were obtained in A20 murine B-cells transfected with the same mlgG BCR constructs (not shown). In these experiments we utilized a tetanus toxin/ovalbumin conjugate and the ovalbumin-specific T-cell hybridoma 3DO.548 to assay antigen presentation (data not shown). [We attribute the slight enhancement seen with the H(KVK) Ramos cells to their somewhat higher levels of BCR expression compared with cells expressing intact mlgG (Figure 5A and data not shown).] Taken together, we conclude that antigen presentation via the mlgG BCR is not measurably compromised by the absence of the γ cytoplasmic tail either in Ramos or in A20 B cells. In contrast, Mel JuSo cells expressing the H(KVK) γ chain (clones 1G9 and 1H5) presented antigen at least 10-fold less effectively compared with cells (clones 5H5 and 5G1) expressing full-length mlgG (Figure 6B and C). This demonstrates that, in the absence of Igα/Igβ, the γct domain is required for maximal
mIgG-mediated antigen presentation. Nonetheless, cells expressing the H(KVK) form of the γ chain still presented antigen substantially better than cells lacking antigen-specific BCRs (Figure 6B and C; also see Discussion). Interestingly, Mel JuSo transfectants expressing the H(Y513A) mutant heavy chain showed a similar loss of antigen presentation efficiency as seen in cells expressing the H(KVK) heavy chain (Figure 6B). Taken together, these results clearly demonstrate, first, that the mIgG BCR delivers equivalent augmentation of antigen presentation in the presence or absence of the Igα/Igβ dimer and, secondly, that the cytoplasmic tail (and in particular Y513) of the γ heavy chain is necessary for full Igα/Igβ-independent antigen presentation capacity.

**Discussion**

Switching of immunoglobulin class, affinity maturation of the antigen binding site and the establishment of immunological memory are all familiar characteristics of the developing antibody-mediated immune response. Less familiar and largely unexplored is a striking change in the structure of the B-cell antigen receptor. Whereas the heavy chains of membrane IgM/D molecules expressed on naive B-cells lack cytoplasmic domains, the class-switched iso-

```plaintext
mIgG-mediated antigen presentation. Nonetheless, cells expressing the H(KVK) form of the γ chain still presented antigen substantially better than cells lacking antigen-specific BCRs (Figure 6B and C; also see Discussion). Interestingly, Mel JuSo transfectants expressing the H(Y513A) mutant heavy chain showed a similar loss of antigen presentation efficiency as seen in cells expressing the H(KVK) heavy chain (Figure 6B). Taken together, these results clearly demonstrate, first, that the mIgG BCR delivers equivalent augmentation of antigen presentation in the presence or absence of the Igα/Igβ dimer and, secondly, that the cytoplasmic tail (and in particular Y513) of the γ heavy chain is necessary for full Igα/Igβ-independent antigen presentation capacity.

**Discussion**

Switching of immunoglobulin class, affinity maturation of the antigen binding site and the establishment of immunological memory are all familiar characteristics of the developing antibody-mediated immune response. Less familiar and largely unexplored is a striking change in the structure of the B-cell antigen receptor. Whereas the heavy chains of membrane IgM/D molecules expressed on naive B-cells lack cytoplasmic domains, the class-switched iso-
```
Mel JuSo cells are negative for surface expression of CD79b (Igβ) and can present antigen to the tetanus toxin-specific T-cell clone KT4 (tetanus toxin 1273–1284; Demotz et al., 1989). (A) Expression of Igα/β (CD79b) on the surface of Ramos (H+L) and Mel JuSo (H+L) transfectants. Igγ heavy and light chain cDNA transfectants (Ramos or Mel JuSo) were stained with the anti-CD79b mAb, CB3-1 (Nakamura et al., 1992) followed with goat anti-mouse FITC or with the goat anti-mouse FITC antibody only (−ve). (B) Untransfected Mel JuSo cells were incubated with graded amounts of tetanus toxin C fragment domain in the presence of the KT4 T cell clone. Proliferation of the clone, measured by [3H]thymidine incorporation (c.p.m.), was measured after 48 h.

Cell surface expression and MIIC targeting of mIgG in Igα/Igβ positive (Ramos) and negative (Mel JuSo) transfectants. (A) Expression of mIgG and MHC class II on the surface of Ramos (H+L), Ramos (H(KVK)+L), Mel JuSo (H+L) and Mel JuSo (H(KVK)+L) transfectants. Igγ heavy and light chain cDNA transfectants (Ramos or Mel JuSo) were stained with the QE11 (anti-human kappa) and L243 (anti-MHC class II) monoclonal antibodies followed with second layers as in Figure 4A. (B) mIgG is found in MIIC-like multivesicular compartments. Mel JuSo transfectants were processed for immunoelectron microscopy as described (West et al., 1994; Lucocq et al., 1995). Cryosections were sequentially labelled for class II MHC using DA6.231 mAb followed by rabbit anti-mouse and small (6 nm) gold, and with sheep anti-human IgG followed by rabbit anti-sheep and large (12 nm) gold. Bars, 200 nm. Note that labelling for mIgG was frequently observed on internal membranes (main figure and inset).

Remarkably, expression of a minimal BCR consisting of only immunoglobulin heavy and light chains boosted antigen presenting capacity at least 1000-fold in the HLA-DR- and DM-positive non-lymphoid cell Mel JuSo, equivalent to the augmentation seen when the same receptor was expressed in Igα/Igβ-positive B-cells. Deletion of γct or mutation of its single tyrosine residue reduced the efficiency of antigen presentation 10-fold in the Igα/Igβ-negative cells. Thus, the mIgG BCR can mediate highly efficient antigen presentation in the absence of Igα/Igβ and the γct domain, and Y513 in particular is required for its full antigen presentation capacity.

Several lines of evidence suggest that endocytosis alone is not sufficient for targeting of antigen (Neibling and Pierce, 1993) or invariant chain complexes (Swier and Miller, 1995) to the compartments where antigen processing and class II MHC maturation take place (reviewed in Watts, 1997). Receptor oligomerization (Mellman and Plutner, 1984; Swier and Miller, 1995) or other targeting mechanisms (Bonnerot et al., 1995; Mitchell et al., 1995) appear necessary for efficient delivery to the processing...
mIgG-mediated antigen presentation

Fig. 6. mIgG augments antigen presentation to the same extent in both Igα/Igβ-positive and -negative cells. 2 × 10^4 irradiated Ramos (A) or Mel JuSo (B and C) clones expressing wild-type or mutant heavy chains plus light chains were co-cultured with 2 × 10^5 T-cells in the presence of graded doses of antigen in 96-well plates. T-cell clones D1c (tetanus toxin 947–966) and KT4 (tetanus toxin 1273–1284; Demotz et al., 1989) were used to detect antigen presentation by Ramos and Mel JuSo respectively. Experiments shown are representative of several and illustrate similar antigen presentation capacity in independently isolated clones expressing the same cDNAs.

compartments. Because mutation of the tyrosine residue in γct reduced the efficiency of antigen presentation 10-fold but did not—at least in the CD4/γct chimera—affect equilibration with peripheral recycling endosomes, we suggest that this residue may also form part of a signal important for post-endocytic targeting of IgG-BCR-bound antigen to the processing and class II MHC loading compartment(s).

We expected that Mel JuSo cells expressing specific antigen receptors lacking the γct domain would still present antigen better than cells lacking BCRs altogether since the latter cells depend solely on fluid phase pinocytosis for antigen entry into the endocytic pathway. However, the degree of enhanced antigen presentation observed in cells expressing the H(KVK) form of the heavy chain was somewhat surprising and raises the interesting possibility that γct is not the sole determinant of antigen targeting in mIgG.

Deletion of the cytoplasmic tail of membrane IgG had no obvious effect on antigen presentation in the two B-cell lines we tested. Presumably, the Igα/Igβ dimer expressed in these cell lines can provide both endocytosis and antigen presentation function for the tailless mIgG as it does for mIgM. This raises the question of the role of γct in class-switched B-cells. Given the high degree of sequence conservation of the cytoplasmic tail among γ sub-types and between species (Bensmana and Lefranc, 1990; Kinoshita et al., 1991) and the fact that it can augment antigen presentation to an extent equivalent to that provided by Igα/Igβ, it seems very likely that this capacity is utilized in vivo following switching to the IgG class. One possibility is that certain IgG-positive B-cells may lose expression either of Igα/Igβ or some other component which interacts with Igα/Igβ to deliver full antigen presentation function to the IgM BCR on naive B-cells. In fact, there is evidence that additional components besides the Igα/Igβ dimer may be important even for IgM class BCRs. Mutation of a tyrosine residue in the transmembrane region of mIgM heavy chain abolished antigen presentation function (Shaw et al., 1990) but did not affect antigen uptake or degradation (Mitchell et al., 1995). Since this mutation did not disrupt association with the Igα/Igβ dimer, these studies indicate that other unidentified components may exist which are crucial for the fidelity of antigen targeting to MHC/CIIV via the IgM BCR. Thus, although expression of the Igα/Igβ dimer apparently persists in IgG-positive B-cells (Venkitaraman et al., 1991; van Noesel et al., 1992) other putative components of the BCR sorting machinery may be poorly expressed or absent in isotype-switched B-cells. Consequently, the switched BCR isotypes may therefore require autonomous antigen presentation capacity as demonstrated here.

Materials and methods

Cell lines and culture conditions

Chinese Hamster Ovary (CHO), HeLa, and Mel JuSo cells were routinely grown at 37°C in Dulbecco’s Modified Eagle’s Medium (DMEM; Gibco-BRL), supplemented with 10% fetal calf serum (FCS), 100 μg/ml kanamycin and 2 mM glutamine. Ramos (human mIgM+, λ B-lymphoma) and A20 (mouse mlgG2a+, κ B-lymphoma) cells were grown in RPMI 1640 (Gibco-BRL) supplemented with 10% FCS, 100 μg/ml kanamycin, 2 mM glutamine, 1 mM pyruvate, non-essential amino acids and 50 μM β-mercaptoethanol. Tetanus toxin-specific T-cell clones, KT4 (donor KK) and D1c (donor PDS) were maintained as previously described (Lanzavecchia, 1985).
mlgG cloning and mutagenesis
5 μg of RNA from the EBV-transformed tetanus toxin-specific B cell clone 11.3 (Lanzavecchia, 1985) was reverse-transcribed with AMV reverse transcriptase (Pharmacia), specifically primed with oligonucleotides (5′-CTGGAGTCTCTAGGGCCTGGCTGATCT-3′ and 5′-CTGAAGGCTCTAACCACCTCTCCCTCCTTGGA-A3′) complementary to the last 18 bp of the human γ1 membrane exon (M2) and the κ constant region respectively (Hieter et al., 1980; Bensmana and Lefranc, 1990; Kinoshita et al., 1991). The primers contained a stop codon and a restriction site. The resulting cDNAs were amplified by PCR using conditions described by Lanzavecchia et al. (1990). The PCR products were cloned into the mammalian expression vectors pMCFR–HphI, pMCFR–H11032, pMCFR–NI or pMCR–pac (kind gifts from Drs Denzin, Cresswell and Novak); their subsequent sequencing confirmed that these products encoded the membrane γ1 heavy and κ light chain products respectively.

For the light chain construct, detectable expression was obtained only after replacing the PCR-amplified leader sequence with that from the γ chain. In addition, residue 512 of the κ heavy chain was changed to aspartic acid because this residue is highly conserved in the Vκ constant regions.

CD4/κ γet chimeric receptor construction
To construct the CD4/κ γet chimeras, plasmids containing either the wild-type or mutant Y513A 11.3 κ cDNAs were mixed with human CD4 cDNA (gift from Dr Mark Marsh) and amplified with the following three oligonucleotides as described (Yon and Fried, 1989): CD4 Avai: 5′-GAGCTCGGGAGAGCTCGTGG-3′; CD4 κ: 5′-GAGAAGAT- CACCTCTACCTAAGAGGTAAGTTCGTACC-3′. Following sequencing, the resulting PCR product was then recloned back into the 11.3 κ CD4 cDNA as described above.

Transfections
Plasmids encoding CD4, CD4/κ γet, CD4/κ γet Y513A, CD4Asp, CD4Δc, 11.3 γ-heavy chain, mutants of γ as indicated and 11.3 light chain were transfected into CHO, MelJuSo and HeLa cells using calcium phosphate precipitation. For the generation of stable clones, ~2.5×10^6 adherent cells were transfected with 10 μg of each plasmid before cloning by limiting dilution with the appropriate selection. Drug-resistant colonies were picked and analysed for expression using immunofluorescence microscopy, FACS analysis or [3H]-labeled tetanus toxin binding. Clones expressing similar levels of transfected product were chosen for subsequent analysis.

For B-cell transfections, 10^7 cells were washed and resuspended in 0.8 ml of PBS containing 100 μg of linear DNA and electroporated at 960 μF, 300 V. After 10 min at room temperature the cells were recultured in RPMI growth medium for 60 h. Cells were then resuspended in 50 ml RPMI growth medium containing the appropriate drug selection and plated out into four 24-well plates (Costar). Clones were screened for surface expression using both FACS analysis and [3H]-labeled tetanus toxin binding. Transfectants used in experiments were representative of several and showed similar levels of cell surface IgG expression.

FACS analysis and immunofluorescence
For FACS analysis, cells were harvested with PBS/10 mM EDTA, washed into PBS/2% FCS and stained with the following. Primary antibodies: biotinylated goat anti-human IgG (Southern Biotech. Assoc. Inc.); L243 (anti-MHC class II; Lampson and Levy, 1980); QEII (anti-human κ chain; Serotec); CB3-1 (anti human CD70; Nakamura et al., 1992). Secondary antibodies: streptavidin FITC; goat anti-mouse FITC (Southern Biotech. Assoc. Inc.). 10,000 events were recorded on a Becton Dickinson FACS.

Transient transfection of HeLa cells was performed as described (Ponnambalam et al., 1994). Briefly, cells were grown to 5–10% confluency on sterile coverslips in 6-well plates (Costar) before transfection with calcium phosphate (~10–15 μg of DNA). 24 h after transfection, cells were washed three times and recultured for a further 48 h before analysis by immunofluorescence microscopy (Ponnambalam et al., 1994). Antibodies were as above and in addition the following primaries: rabbit anti-human IgG (Southern Biotech. Assoc. Inc.); sheep anti-human IgG (Scottish Antibody Production Unit). Secondaries: as above and in addition: goat anti-rabbit Texas Red (Vector); donkey anti-sheep Texas Red and donkey anti-mouse FITC ( Jackson). Microscopy was performed on a Nikon Microphot-SA attached to an MRC 600 series confocal scanning microscope.

Internalization assays
Approximately 2.5×10^6 CHO transfectants/well were plated in 6-well plates (Costar) 48 h before the assay. The CD4-specific mAb Q4120 (gift from the MRC AIDS Directed Programme Reagent Project) was labelled to a specific activity of 7.5×10^4 c.p.m./ng using water-soluble [35S]-labelled Bolton Hunter reagent (Amersham). Cells were incubated with 2–3 nM [35S]-labelled Q4120 (1.5×10^6 c.p.m.) in RPMI/HEPES/1% FCS for 1 h at 4°C. After washing, cells were incubated at 37°C for various times in 1 ml of media. At the end of each time point the percentage of ligand endocytosed was quantitated (LKB Multigamma 1261) following two cycles of acid-stripping (1 ml of 150 mM HCl/1% FCS for 1 h at 4°C). After washing, one half of each sample was treated with 1 μl endoglycosidase H (Boehringer-Mannheim) for 16 h at 37°C before SDS–PAGE analysis.

Antigen presentation assays
2×10^4 irradiated MelJuSo or Ramos cells were co-cultured with 2×10^4 T-cells in the presence of graded doses of antigen (His-tagged tetanus toxin C-fragment domain; residues 865–1315) in 96-well flat-bottomed plates as described (Lanzavecchia, 1985). T-cell proliferation was measured after 48 h by [3H]thymidine (Amersham) pulsing (16 h; 1 μCi) and liquid scintillation counting. T-cell clones KF1 (tetanus toxin 1273–1315), TBR1 (tetanus toxin 1273–1284; Demotz et al., 1989) and D1c (tetanus toxin 947–976) were used to detect antigen presentation by Mel JuSo and Ramos cells respectively.

Acknowledgements
We thank C.Borrebbeck, P.Dellabona, P.J.Dyson, J. Ellis and M. Roth for advice; A.Lanzavecchia, M.Marsh, D.Mason, J.Borst, C.–A.Siegrist, E.Hewitt, T.Novak, P.Cresswell, C.Rooney, B.Spruce and H.Holmes for reagents; and J.Lames, M.L.Kay and L.Grayson for technical assistance. We are particularly grateful to A.Antoniou for A20 transfectants and E.Smythe for comments on the manuscript. This work was supported by the Wellcome Trust.

References
mIgG-mediated antigen presentation


Received on February 12, 1997; revised on March 26, 1997