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Isotype-specific cross-linking of select human FcγR isoforms triggers release of IL-6

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SUMMARY

Anti-CD3 MoAbs are widely used in T cell activation studies, and are effective in immunosuppressive therapy. We used a panel of mouse (m) anti-CD3 switch variant MoAbs of five different isotypes to study IL-6 release from accessory cells. Incubation of human (h) mononuclear cells with anti-CD3 MoAbs resulted in increased IL-6 levels with MoAbs of mIgG1 and mIgG2a isotypes, with no effect of mIgG2b or mIgA. This suggested involvement of IgG Fc receptors (FcγR) in triggering IL-6 production. To evaluate the role of different FcγR molecules individually we used a panel of hFcγR-transfected mouse fibroblasts, and Jurkat T cells as a model. IL-6 secretion by CD32 transfectants expressing the hFcγRIIa high-responder (HR) allelic form was triggered by mIgG1 anti-CD3 MoAb, with no effect of four other isotypes. None of the anti-CD3 MoAbs induced IL-6 secretion by CD32 transfectants expressing either a variant of this receptor, containing only a single intracellular amino acid (CT-), the hFcγRIIa low-responder (LR) allelic form, or hFcγRIIb1. hFcγRI (CD64) transfectants exhibited IL-6 production after incubation with mIgG2a anti-CD3 MoAb, and to a lesser extent with mIgG2b, and mIgG1 MoAb. Indirect involvement of T cells in triggering IL-6 secretion could be excluded by experiments in which transfectants were cultured with immobilized anti-CD3 MoAb. These data indicate that cross-linking of either hFcγRI, or hFcγRIIa^{HR} by appropriate anti-CD3 MoAbs triggers IL-6 production of accessory cells, and not T cells. This may also take place *in vivo* during immunosuppressive therapy with anti-CD3 MoAbs, and related antibody-mediated immune responses.

Keywords IL-6 FcγR CD32 CD64 transfectants

INTRODUCTION

The CD3 complex plays a central role in T cell activation, is associated with α, β or γ, δ T cell receptors (TCR) and is crucial for signal transduction. Anti-CD3 MoAbs can activate T cells in the absence of antigen and have, therefore, been widely used in T cell activation studies [1,2]. MoAbs directed against the CD3 antigen, furthermore, proved effective in immunosuppressive therapy after organ transplantation [3,4]. However, cross-linking of anti-CD3 MoAb by Fc receptors on accessory cells (e.g. monocytes) is essential for T cell activation to occur [5,6].

Receptors recognizing the Fc-region of immunoglobulin (FcR) play a critical role in several immune processes like phagocytosis, antibody-dependent cellular cytotoxicity, clearance of immune complexes, and secretion of inflammatory mediators and reactive oxygen intermediates. Human leucocyte FcR for IgG (FcγR) can be divided into three classes: human (h) FcγRI (CD64), hFcγRII (CD32), and hFcγRIII (CD16). This distinction is based on molecular weight, cellular distribution, MoAb reactivity, and cDNA and gene structure [7–9]. hFcγRI (for which recently three genes have been described [10,11]), is primarily expressed on mononuclear phagocytes, shows high affinity for monomeric hIgG1 and hIgG3 isotypes, and cross-reacts with mouse (m) IgG2a, and mIgG3. hFcγRII exhibits low affinity for IgG, has a broad distribution pattern, and is expressed on virtually all types of leucocytes. Furthermore, multiple isoforms have been described, encoded by three genes (hFcγRIIA, B, and C) [7–9,12]. hFcγRIIA is furthermore polymorphic, and monocytes from different individuals exhibit either a strong (HR individuals) or a weak (LR individuals) interaction with mIgG1 complexes [6,13,14]. Interestingly, LR molecules (hFcγRIIa^{LR}) can efficiently interact with hIgG2 dimers, in contrast to FcγRIIa^{HR} molecules [15,16]. Recently, the molecular basis for this polymorphism has been attributed to a single amino acid substitution at position 131 in the hFcγRIIa molecule [16–18].

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In addition to Fc receptor cross-linking, production of cytokines (e.g. IL-1) is required for T cell activation by anti-CD3 MoAb [19,20]. A recent study showed production of IL-6 during this process [21]. Lymphoproliferation studies showed T cell activation by MoAb to be strictly dependent on the isotype of anti-CD3 MoAb. In the present study we analysed IL-6 production during anti-CD3-induced T cell mitogenesis in detail. FcγR on monocytes and a set of FcγR-transfectants were found to play a critical role in this process, as shown by studies using a panel of mouse anti-CD3 switch variant MoAbs.

MATERIALS AND METHODS

Antibodies

mIgG1,κ MoAb CLB-T3/4.1 and its heavy chain isotype variants recognizing the ε chain of the TCR/CD3 complex, have been described before [22]. No detectable endotoxin levels (< 1 pg/ml) could be measured in these MoAb preparations with a chromogenic Limulus assay (Kabivitrum, Stockholm, Sweden). To exclude endotoxin contamination of media and sera, these antibodies were tested for their capacity to stimulate IL-6 production by monocytes [23]; none of them induced detectable IL-6 production (< 5 U/ml). The anti-FcγRI MoAbs 32 [24], 197, and 22 [25] (all purified IgG), and anti-FcγRII MoAb IV3 [26] (purified IgG) were from Medarex (Lebanon, NH). Anti-FcγRII MoAb AT10 was kindly provided by Dr M. Glennie (Tenovus Research Laboratory, Southampton, UK; [27]). A polyclonal goat anti-rhIL-6 antiserum selectively neutralizing human IL-6 activity, and not mouse IL-6, was prepared as described [28]. Rat anti-mouse IL-6 MoAb MP5-20F3 [29] was a kind gift of Dr J. Abrams (DNAX, Palo Alto, CA).

FcγR-transfectants

To obtain stable hFcγRI transfectants, 3T3 fibroblasts were transfected with pDOL$^-$ (retroviral expression vector) containing hFcγRI cDNA p135 [30]. FACS sorting, performed by Dr P. Guyre (Dartmouth Medical School, Hanover, NH) was used to isolate transfectants with high hFcγRI expression levels. Expression of hFcγRI was determined by staining with MoAbs 32 and 22. Mouse 3T6 cells transfected with hFcγRIIa^{HR} cDNA pCD-16.2 [31], hFcγRIIa^{CT-} cDNA pCD-16.2 [32], pcDX containing hFcγRIIa^{LR} cDNA pPW3 [33], or pCDM8 containing hFcγRIIb1 cDNA pIP14^2 [33a] have been described before [33,34]. The hFcγRIIb1 cDNA is identical to those described before [35], except for one amino acid change (tyrosine into aspartic acid at position 11 of the cytoplasmic tail). The pCD-16.2 and pCD-16.2 FcγRIIa^{CT-} cDNAs were generous gifts of Dr K. Moore (DNAX).

Cell culture

Jurkat, mouse 3T3 and mouse 3T6 cells were cultured in RPMI 1640 medium containing 10% fetal calf serum (FCS). Stable transfectants were cultured in RPMI 1640 medium supplemented with 5% FCS and antibiotics.

IL-6 bioassay

IL-6 activity of supernatants was determined by the B9 bioassay [36]. Briefly, 5000 B9 cells were cultured in flat-bottomed microtitre wells (Nunc, Roskilde, Denmark). After 64 h, cells were labelled with 0·5 μCi methyl ^{3}H-thymidine. Samples were titrated in two-fold dilutions and related to a standard. One Unit/ml (= 1 pg/ml) is defined as the concentration inducing half-maximal thymidine incorporation in B9 cells.

Immunofluorescence

Fibroblasts (2–5 × 10^5 cells/test) were incubated with MoAb (appropriately diluted in PBS containing 1% bovine serum albumin (BSA) and 0·1% NaN_3) for 30 min at 4°C. After washing, cells were incubated with FITC-labelled F(ab′)$_2$ fragments of goat anti-mouse immunoglobulin (H&L) antiserum (Tago, Burlingame, CA) for 30 min at 4°C. Following washing twice, cells were fixed with 1% paraformaldehyde (v/v), and fluorescence was quantified using a FACStar (Becton Dickinson, Mountain View, CA). Fluorescence data of 5000 cells are expressed as percentage fluorescent cells corrected for background fluorescence (incubated with conjugate only). Expression on hFcγRI transfectants was determined by using anti-CD64 MoAb 22, and that of hFcγRII transfectants either by anti-CD32 MoAb IV3 (for hFcγRIIa$^{HR/LR/CT-}$), or by MoAb AT10 (for hFcγRIIb1) reactivity. Quantification of the number of FcγR molecules expressed on transfectants was performed using quantitative fluorescein standards and beads with a known number of immunoglobulin-binding sites (Flow Cytometry Standards Corporation, Research Triangle Park, NC), as described previously [16].

Antibody stimulation

Peripheral blood mononuclear cells (PBMC) were isolated as described [22]. For antibody stimulation experiments 2 × 10^5 PBMC in 200 μl were cultured in flat-bottomed microtitre plates (Nunc) in ultrafiltrated Iscove's modified Dulbecco's medium (IMDM) with 5% (specially selected) lipopolysaccharide (LPS)-free FCS, and endotoxin-free anti-CD3 MoAbs were added. No detectable endotoxin levels of IMDM medium could be measured either by the Limulus assay, or after monocyte incubation (see above). After culturing for 24 h, supernatants were harvested and tested for IL-6 activity.

Transfected mouse fibroblasts, cultured to near confluency in tissue culture flasks, were incubated with IL-1β (10 U/ml; Boehringer Mannheim), or with Jurkat cells (0·5 × 10^6/ml) and anti-CD3 MoAb (2 μg/ml) for 24 h. Supernatants were then harvested and relative IL-6 levels determined. Relative IL-6 levels represent:

$$\frac{\text{IL-6 levels in supernatants}}{\text{IL-6 levels in IL-1}\beta\text{-stimulated fibroblast supernatants}} \times 100\%$$

In some of the experiments anti-CD3 MoAbs were diluted in PBS (pH 7·4) and coated overnight (100 μg/ml) at 4°C in 24-well tissue culture plates (Costar). After two washing steps with PBS (to remove unbound MoAb), transfectants were added to the wells in 500 μl RPMI 1640 medium with 5% FCS. Supernatants were harvested after 24 h and relative IL-6 levels were determined.

Adherence assays

The number of adhering cells to immobilized complexes was quantified after 24 h and compared with that of transfectants adhering to uncoated wells. At least 200 cells were scored per test. To show FcγR-dependency of adherence, transfectants were incubated with or without anti-CD64 MoAb 197 (10 μg/ml) or anti-CD32 MoAb IV3 (10 μg/ml) for 30 min at 4°C before addition to macrowells coated with MoAb.

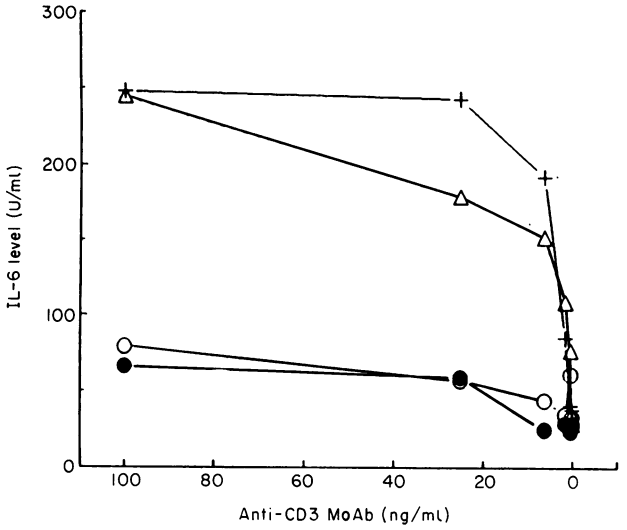

Fig. 1. Effect of murine anti-CD3 switch variant MoAb on IL-6 secretion by peripheral blood mononuclear cells (PBMC). Mononuclear cells were cultured with different amounts of anti-CD3 switch variant MoAb of either the mIgG1 (+), mIgG2a (△), mIgG2b (○), or mIgA (●) isotypes as described in Materials and Methods. After 24 h, IL-6 activity of supernatants was determined in the B9-assay. Experiment was repeated twice using cells from different donors with similar results.

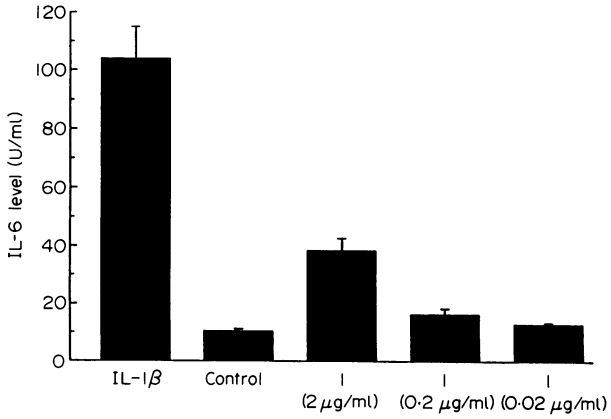

Fig. 2. mIgG1 anti-CD3 MoAb-induced IL-6 production by hFcγRIIa^{HR} transfectants. hFcγRIIa^{HR} transfectants were incubated for 24 h with IL-1β (10 U/ml) alone, or with varying amounts of mIgG1 anti-CD3 MoAb in the presence of Jurkat cells. IL-6 levels in supernatants were determined as described in Materials and Methods. Control represents transfectants cultured with Jurkat cells only. Results (mean ± s.d. of duplicates) are representative for two individual experiments.

Coating efficiency

Coating efficiencies of anti-CD3 MoAb to culture plates were determined by a mouse immunoglobulin-specific ELISA, according to manufacturer's recommendations (Nordic, Tilburg, The Netherlands).

RESULTS

Mouse anti-CD3 switch variant MoAbs have recently been used to analyse immunoglobulin-isotype dependency of anti-CD3-induced T cell proliferation [15]. The anti-CD3 MoAbs used recognize a single epitope with similar affinity but differ in their C_H chains [22]. These same MoAbs were used to analyse IL-6 production during anti-CD3-induced T cell activation. This was done by culturing PBMC with varying doses of murine anti-CD3 isotype switch variant MoAb. Significantly increased IL-6 levels were induced by mIgG1 and mIgG2a anti-CD3 MoAb, whereas mIgG2b and mIgA had only marginal effects (Fig. 1). The effects on IL-6 levels were, furthermore, dose-dependent (Fig. 1), and the isotype specificity pointed at FcγR involvement in this process. In accordance with a recent report [21], our data suggested FcγRI (CD64) involvement, as increased IL-6 levels were induced by a mIgG2a anti-CD3 MoAb, a subclass which interacts effectively with CD64. The enhanced IL-6 levels resulting from incubation with mIgG1 anti-CD3 MoAb, furthermore, pointed at involvement of FcγRII (CD32) [7–9].

To determine the role of FcγR in anti-CD3-induced IL-6 production in more detail, the PBMC system seemed unsuitable. The accessory cells (mainly monocytes) in this system co-express FcγRI and FcγRII [7–9] and the role of each individual receptor can not, therefore, be determined unequivocally. Furthermore, the possibility of simultaneous and/or synergistic interactions for different classes of FcγR in signalling [37–39], does not allow a clear interpretation of results obtained with PBMC. More importantly, T cells have also been found capable of producing IL-6 [40], and therefore may mediate the IL-6 production reported for PBMC.

Fibroblasts, however, are well known IL-6 producers [40], and cells transfected with FcγR cDNA proved to be excellent accessory cells in anti-CD3-induced T cell proliferation studies [15,32]. Making use of a panel of murine transfectants expressing distinct hFcγR cDNAs, and Jurkat cells (a human T cell line which does not produce hIL-6 upon triggering; data not shown), we addressed the role of individual hFcγRI and hFcγRII molecules. Furthermore, the use of murine fibroblasts and human T cells enabled us to determine the cellular source of anti-CD3-induced IL-6 more precisely.

Transfected mouse 3T6 cells expressing high numbers ($150 \pm 30 \times 10^3$ molecules/cell; $n = 3$) of hFcγRIIa^{HR}, were co-cultured with CD3$^+$ Jurkat cells in the presence of varying amounts of mIgG1 anti-CD3 MoAb (Fig. 2). IL-6 levels in supernatants were compared with IL-6 levels obtained by stimulating cells with IL-1β, a powerful inducer of IL-6 production by fibroblasts [41]. In the presence of mIgG1 MoAb, the hFcγRIIa^{HR} transfectants produced significant amounts of IL-6, which was, furthermore, dose-dependent. The hFcγRIIa^{HR} transfectants were identified as the source of IL-6 by inhibition studies using a MoAb directed against mouse IL-6, which completely inhibited IL-6 activity in the supernatants. Furthermore, a polyclonal antiserum directed against human IL-6 did not inhibit IL-6 activity in the B9-hybridoma cell assay (Fig. 3). In parallel, IL-6 activity in a control sample containing 400 U/ml rhIL-6 was completely neutralized by this last antiserum. As the B9 assay is known to be (equally) sensitive to mouse IL-6 and human IL-6 [36], these experiments strongly support the IL-6 found in cultures to be of murine origin.

As hFcγRIIa^{HR} transfectants were found capable of IL-6 production upon culture with mIgG1 anti-CD3 MoAb, we set out to examine FcγR involvement in more detail. hFcγRIIa^{HR} and hFcγRIIa^{LR} allotypic forms are identical except for two amino acid substitutions at positions 27 (Q to W) and 131 (R to H) in the extracellular domain. The latter substitution was

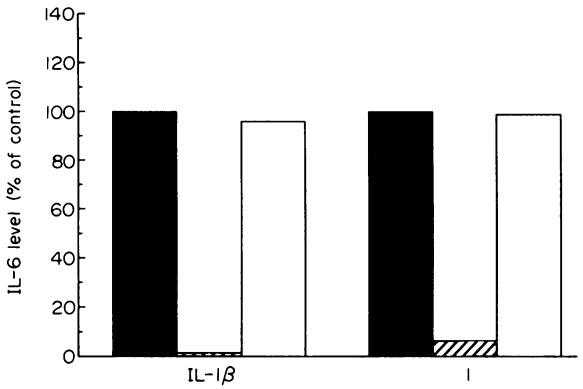

Fig. 3. Source of IL-6 in supernatants. hFcγRIIa^{HR} transfectants and Jurkat cells were incubated with IL-1β (10 U/ml), or with mIgG1 anti-CD3 MoAb for 24 h. The origin of IL-6 found in supernatants was determined by performing the B9 bioassay in the presence of either a neutralizing MoAb directed against mouse IL-6 (▨), or a polyclonal antiserum with specificity for human IL-6 (□), and related to IL-6 levels determined in media without anti-IL-6 antibodies (■). Experiment was repeated once with similar results.

found critical in determining either an efficient (HR) or inefficient (LR) reactivity with mIgG1 complexes [16,18]. Transfectants expressing high numbers of hFcγRIIa^{HR}, or hFcγRIIa^{LR} molecules (Fig. 4a) were analysed for IL-6 production triggered upon addition of anti-CD3 MoAb of different isotypes in the presence of Jurkat cells. Anti-CD3 MoAb of the mIgG1 isotype enhanced IL-6 production, above background levels, in cultures with FcγRIIa^{HR}-, but not FcγRIIa^{LR}-expressing transfectants (Fig. 4b). We simultaneously tested a transfectant which expressed a variant hFcγRIIa^{HR} molecule containing only a single intracellular amino acid (FcγRIIa$^{CT^-}$; Fig. 4a). Although hFcγR expression and levels of IL-6 induced by IL-1β were comparable for all three types of cells, none of the anti-CD3 MoAbs induced significant increased IL-6 levels with the FcγRIIa^{LR} or FcγRII$^{CT^-}$ transfectants. Increased IL-6 levels were not observed on culture of mock-transfected 3T6 cells with any of these anti-CD3 MoAbs (at 2 μg/ml; data not shown).

Transfectants expressing high levels of the high-affinity hFcγRI receptor, and one of the hFcγRIIB-gene products, hFcγRIIb1 (Fig. 5a), an isoform preferentially expressed in B cells [8], were also investigated. Significant IL-6 production was found in supernatants of cultures with hFcγRI transfectants, Jurkat cells and mIgG2a anti-CD3 MoAb (Fig. 5b). Low, but significant levels were also found when using mIgG2b or mIgG1 anti-CD3 MoAb (Fig. 5b). The hFcγRIIb1 transfectants, in spite of having high hFcγRIIb1 expression, were unable to produce IL-6 upon culture with any of the anti-CD3 MoAbs tested (Fig. 5b), despite similar levels of IL-6 triggered upon addition of IL-1β.

To assess the role of the Jurkat T cells we studied induction of IL-6 production by FcγR-transfectants triggered via solid phase-bound moAb anti-CD3. Similar isotype-specific effects were observed when hFcγRI or hFcγRIIa^{HR} transfectants were incubated in macrowells coated with equivalent amounts of MoAb of various isotypes. Immobilized mIgG2a, and not mIgG2b induced IL-6 production from hFcγRI transfectants (Fig. 6). Similarly, immobilized mIgG1 triggered release from hFcγRIIa^{HR} transfectants (relative IL-6 level: 28±6; $n=2$).

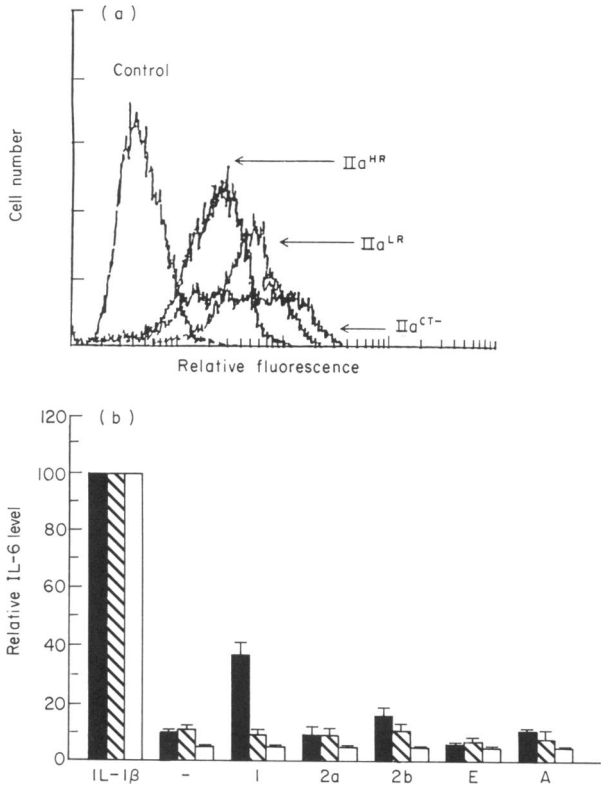

Fig. 4. Triggering of IL-6 production from hFcγRIIa transfectants by murine anti-CD3 switch variant MoAb. Murine 3T6 fibroblasts with high expressions of hFcγRIIa^{HR}, hFcγRIIa^{LR}, and hFcγRIIa$^{CT^-}$ as assayed with anti-CD32 MoAb IV3 (a), were incubated with different murine anti-CD3 switch variant MoAbs in the presence of Jurkat cells. Relative IL-6 levels of supernatants (b) were determined as described in Materials and Methods (mean±s.d. of duplicates). Experiment was repeated twice with similar results. Control in (a) represents untransfected cells incubated with MoAb IV3. Control in B (−) represents transfectants cultured with Jurkat cells only. Absolute amounts of IL-1β-induced IL-6 by transfectants: hFcγRIIa^{HR}, 104 U/ml; hFcγRIIa^{LR}, 102 U/ml; hFcγRIIa$^{CT^-}$, 122 U/ml. ■, IIa^{HR}; ▧, IIa^{LR}; □, IIa$^{CT^-}$.

FcγR specificity of IL-6 production from FcγRI-transfected cells was shown by abrogation of IL-6 production observed in the presence of anti-FcγRI MoAb 197 (or anti-FcγRII MoAb IV3 for FcγRIIa^{HR} cells; data not shown). The isotype specificity of the hFcγRI transfectant was, furthermore, reflected in the attachment of transfectants to macrowells coated with equivalent amounts of anti-CD3 MoAb of different isotypes. In plates coated with 100 μg of mIgG2a or mIgG2b MoAb, we found 30±5% ($n=2$) transfectants binding to mIgG2a, and 10±5% ($n=2$) to mIgG2b MoAb. Attachment was, furthermore, hFcγRI-dependent, as indicated by decreased binding in the presence of the blocking anti-hFcγRI MoAb 197 (6±6%; $n=2$). Similar results were obtained with hFcγRIIa^{HR} transfectants, using mIgG1- and mIgG2b-coated plates, with decreased binding in the presence of MoAb IV3 (data not shown). These controls, furthermore, supported that transfectants constituted the source of IL-6 produced upon triggering with anti-CD3 MoAb, and argue against a role for T cell-mediated cytokines in this process.

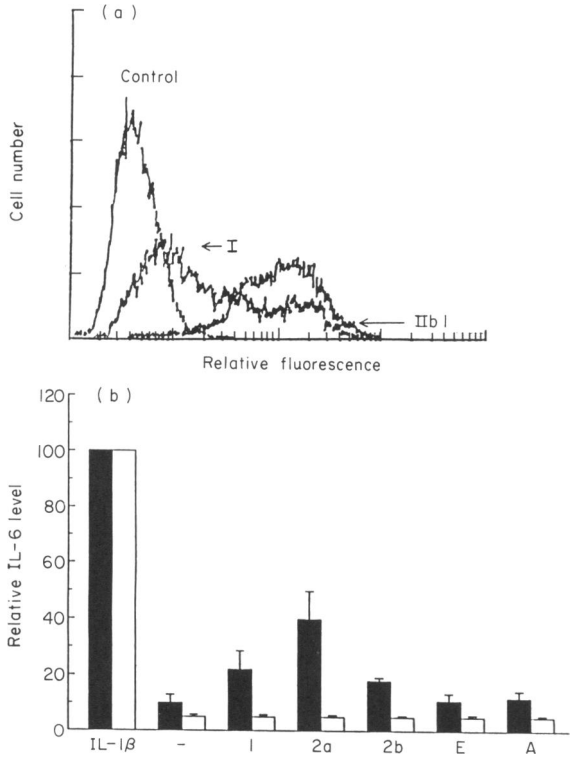

Fig. 5. Triggering of IL-6 production from hFcRI, and hFcγRIIb1 transfectants by murine anti-CD3 switch variant MoAb. Murine transfectants with high expressions of hFcγRI or hFcγRIIb1 as assayed with anti-CD64 MoAb 22 (hFcγRI), or anti-CD32 MoAb AT10 (hFcγRIIb1), respectively (a), were incubated with different murine anti-CD3 switch variant MoAbs in the presence of Jurkat cells. Relative IL-6 levels of supernatants (b) were determined (mean ± s.d. of duplicates). Experiment was repeated twice yielding almost identical results. Control in (a) represents untransfected cells incubated with MoAb 22. Control in B (−) represents transfectants cultured with Jurkat cells only. Absolute amounts of IL-1β-induced IL-6 by transfectants: hFcγRI, 133 U/ml; hFcγRIIb1, 215 U/ml. ■, I; □, IIb1.

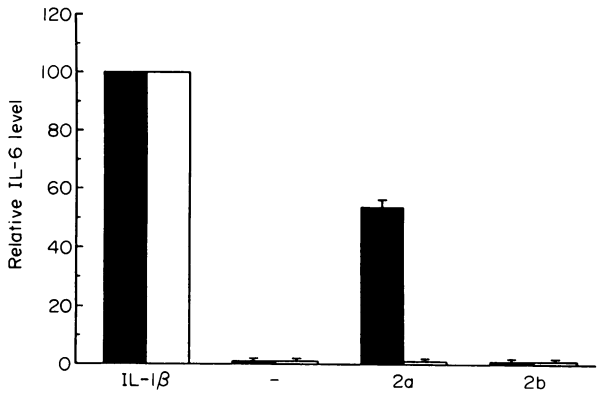

Fig. 6. Immobilized mIgG2a MoAb triggers IL-6 secretion from hFcγRIa transfectants. hFcγRIa-transfectants were preincubated in medium alone (control; IL-1β-induced IL-6 level, 192 U/ml), or medium with anti-CD64 MoAb 197 (IL-1β-induced IL-6 level, 152 U/ml) for 30 min at 4°C. Cells were washed and cultured in uncoated plates (−), or plates coated either with 100 μg mIgG2a (2a), or 100 μg mIgG2b (2b) anti-CD3 switch variant MoAb. Relative IL-6 levels of supernatants were determined after 24 h. Results (mean ± s.d. of duplicates) are representative for two individual experiments. ■, −; □, MoAb 197.

DISCUSSION

In this study significant IL-6 production was observed after incubation of PBMC with mouse anti-CD3 MoAb switch variants. This IL-6 production was isotype-specific and only mIgG1 and mIgG2a induced measurable amounts of IL-6 in PBMC. These results suggested hFcγRI (CD64) involvement, as this receptor has a high affinity for mIgG2a. Furthermore, the results obtained with mIgG1 suggested a role for hFcγRII (CD32). hFcγRII has been shown to exhibit affinity for mIgG1 complexes [7–9]. In order to address the involvement of each FcγR isoform individually, without potential complications caused by interactions between different FcγR classes, or IL-6-producing T cells, we made use of a panel of hFcγR transfectants in murine cells and (human) Jurkat T cells.

The results obtained with FcγR-transfected mouse fibroblasts were in accordance with those obtained using monocytes, and show the capacity of both hFcγRIa and hFcγRIIa^{HR} to trigger IL-6 production from FcγR-positive cells. Furthermore, the data obtained with the hFcγRIIa^{HR} and hFcγRIIa^{LR} transfectants are in line with those obtained in anti-CD3 T cell proliferation studies using these transfectants [15]. In this last report we showed the capacity of hFcγRIIa^{HR} transfectants to solely support mIgG1-induced T cell proliferation, whereas cells expressing hFcγRIIa^{LR} were unable to support T cell mitogenesis with anti-CD3 MoAb of mIgG1, 2a, 2b, or 3 isotypes. The lack of IL-6 production found with the hFcγRIIa$^{CT^-}$ transfectants points at involvement of the FcγR cytoplasmic tail in triggering induction of IL-6 from 3T6 fibroblasts. In T cell proliferation, however, mere cross-linking of hFcγRII seems sufficient (with no necessity for the cytoplasmic receptor region [32]). The importance of a (correct) cytoplasmic tail for triggering of IL-6 production is further accentuated by the lack of IL-6 production observed with hFcγRIIb1 transfectants using mIgG1, mIgG2a, and mIgG2b anti-CD3 MoAbs. These same antibodies, however, could all effectively trigger T cell proliferation with these transfectants as accessory cells [33a].

Krutmann *et al.* did not observe stimulation of IL-6 production in PBMC by mIgG1 MoAb, and suggested an inability of hFcγRII to trigger IL-6 secretion [21]. A likely explanation for the apparent discrepancy with the present data may be varying plate coating efficiencies between the MoAb used by these last authors, resulting in suboptimal cross-linking by mIgG1, as compared with mIgG2a. Furthermore, these authors determined IL-6 levels of supernatants 4 h after stimulation with MoAb. The kinetics of IL-6 induction, however, may vary for the different FcγR, with 4 h being too early to detect any IL-6 induction by hFcγRII cross-linking. Finally, in this last study, control PBMC already showed high IL-6 levels after 4 h, probably due to endotoxin contamination, which may have obscured any IL-6 induction triggered via hFcγRII cross-linking. Our PBMC experiments were performed in ultrafiltered, endotoxin-free media with endotoxin-free anti-CD3 MoAb and, therefore, endotoxin side effects are very unlikely (IL-6 activity found in media from unstimulated PBMC after 24 h: 29 U/ml (Fig. 1); $n=2$).

FcγR are not only involved in triggering IL-6 production but can also mediate release of other inflammatory cytokines. Debets *et al.* presented results showing induction of tumour

necrosis factor-alpha (TNF-α) release from monocytes after cross-linking of hFcγRI or hFcγRII [42]. The present data, furthermore, show that transfected fibroblasts may be excellent tools to study FcγR-mediated triggering, and enabled us to show unambiguously that cross-linking of Fc receptors on accessory cells can result in IL-6 production during *in vitro* T cell activation. This production can be mediated by hFcγRI, or hFcγRIIa^{HR} upon triggering by suitable MoAbs. The fact that cross-linking of FcγR triggers IL-6 secretion was further supported by similar results obtained with substrate-immobilized immunoglobulin of specific isotypes. These last data also excluded an (indirect) role of Jurkat T cells in the secretion of IL-6 from fibroblasts. Notably, in these experiments mIgG2b did not show any significant triggering of IL-6 release, while the same MoAb was found capable of inducing low (albeit significant) levels of IL-6 from the same transfectants in the experiments using Jurkat cells (Fig. 5). Although the basis for this difference is unclear, we feel that the latter way of presenting mIgG2b Fc-domains to FcγRI may be more effective than the 'non-specific' coating (used in Fig. 6).

The present results may have clinical implications for the *in vivo* use of murine anti-CD3 MoAb as immunosuppressives after organ transplantation. *In vivo* experiments in chimpanzees showed absence of side effects using a mIgA switch variant of a mIgG1 anti-CD3 MoAb compared with a mIgG2a switch variant [43]. Use of the mIgG2a anti-CD3 MoAb itself was accompanied by (severe) side effects, paralleled by significant rises in IL-6 and interferon-gamma (IFN-γ) 6 h after administration. Cytokines may well be responsible for the systemic reactions observed in human patients upon receiving anti-CD3 MoAb. Until now, these cytokine-induced effects were thought to result from T cell activation and/or lympholysis [44,45]. The results presented here show that cross-linking of select FcγR isoforms on accessory cells also triggers cytokine production. It is intriguing to speculate that the selective use of isotypes (such as IgE, IgA) may prevent triggering of cytokine release via FcR on accessory cells and thereby limits/reduces cytokine-related side effects in patients.

REFERENCES

1 Clevers H, Alarcon B, Wileman T, Terhorst C. The T cell/CD3 complex: a dynamic protein ensemble. Annu Rev Immunol 1988; **6**:629–62.

2 Altman A, Coggeshall KM, Mustelin T. Molecular events mediating T cell activation. Adv Immunol 1990; **48**:227–360.

3 Ortho Multicenter Transplant Study Group. A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. N Engl J Med 1985; **313**:337–42.

4 Kovarik J, Zlabinger GJ, Wolfram H *et al.* OKT3 monoclonal antibody treatment in renal transplant recipients: clinical experience in 49 patients. Transplant Proc 1989; **21**:1748–52.

5 Kaneoka H, Perez-Rojas G, Sasasuki T, Benike CJ, Engleman EG. Human T lymphocyte proliferation induced by a pan-T monoclonal antibody (anti-Leu 4): heterogeneity of response is a function of monocytes. J Immunol 1983; **131**:158–64.

6 Tax WJ, Hermes FF, Willems RW, Capel PJA, Koene RA. Fc receptors for mouse IgG1 on human monocytes: polymorphism and role in antibody-induced T cell proliferation. J Immunol 1984; **133**:1185–9.

7 Van de Winkel JGJ, Anderson CL. Biology of human IgG Fc receptors. J Leukocyte Biol 1991; **49**:511–24.

8 Ravetch JV, Kinet JP. Fc receptors. Annu Rev Immunol 1991; **9**:457–92.

9 Unkeless JC. Function and heterogeneity of human Fc receptors for immunoglobulin G. J Clin Invest 1989; **83**:355–61.

10 Van de Winkel JGJ, Ernst LK, Anderson CL, Chiu IM. Gene organization of the human high affinity receptor for IgG, FcγRI (CD64). J Biol Chem 1991; **266**:13449–55.

11 Ernst LK, van de Winkel JGJ, Chiu IM, Anderson CL. Three genes for the human high-affinity Fc receptor for IgG (FcγRI) encode four distinct transcription products. J Biol Chem 1992; **267**:15692–700.

12 Qiu WQ, de Bruin D, Brownstein BH, Pearse R, Ravetch JV. Organization of the human and mouse low-affinity FcγR genes: duplication and recombination. Science 1990; **248**:732–5.

13 Tax WJM, Willems HW, Reekers PPM, Capel PJA, Koene RAP. Polymorphism in mitogenic effect of IgG1 monoclonal antibodies against T3 antigen on human T cells. Nature 1983; **304**:445–7.

14 Van de Winkel JGJ, Tax WJM, van Bruggen MCJ *et al.* Characterization of two Fc receptors for mouse immunoglobulins on human monocytes and cell lines. Scand J Immunol 1987; **26**:663–72.

15 Parren PWHI, Warmerdam PAM, Boeije LCM, Capel PJA, van de Winkel JGJ, Aarden LA. Characterization of IgG FcR-mediated proliferation of human T-cells induced by mouse and human anti-CD3 monoclonal antibodies; identification of a functional polymorphism to human IgG2 anti-CD3. J Immunol 1992; **148**:695–701.

16 Warmerdam PAM, van de Winkel JGJ, Vlug A, Westerdaal NAC, Capel PJA. A single amino acid in the second Ig-like domain of the human Fcγ receptor II is critical for human IgG2 binding. J Immunol 1991; **147**:1338–43.

17 Clark MR, Clarkson SB, Ory PA, Stollman N, Goldstein IM. Molecular basis for a polymorphism involving Fc receptor II on human monocytes. J Immunol 1989; **143**:1731–4.

18 Clark MR, Stuart SG, Kimberly RP, Ory PA, Goldstein IM. A single amino acid distinguishes the high-responder from the low-responder form of Fc receptor II on human monocytes. Eur J Immunol 1991; **21**:1911–6.

19 Schwab R, Crow MK, Russo C, Weksler ME. Requirements for T cell activation by OKT3 monoclonal antibody: role of modulation of T3 molecules and interleukin 1. J Immunol 1985; **135**:1714–8.

20 Meuer SC, Meyer zum Buschenfelde KH. T cell receptor triggering induces responsiveness to interleukin 1 and interleukin 2 but does not lead to T cell proliferation. J Immunol 1986; **136**:4106–12.

21 Krutmann J, Kirnbauer R, Köck A *et al.* Cross-linking Fc receptors on monocytes triggers IL-6 production. J Immunol 1990; **145**: 1337–42.

22 Van Lier RAW, Boot JHA, de Groot ER, Aarden LA. Induction of T-cell proliferation with anti-CD3 switch-variant monoclonal antibodies: effects of heavy-chain isotype in monocyte-dependent systems. Eur J Immunol 1987; **17**:1599–604.

23 Aarden L, Helle M, Boeije L, Pascual-Salcedo D, de Groot E. Differential induction of interleukin-6 production in monocytes, endothelial cells and smooth muscle cells. Eur Cytokine Net 1991; **2**:115–20.

24 Anderson CL, Guyre PM, Within JC, Ryan DH, Looney RJ, Fanger MW. Monoclonal antibodies to Fc receptors for IgG on human mononuclear phagocytes. J Biol Chem 1986; **261**:12856–64.

25 Guyre PM, Graziano RF, Vance BA, Morganelli PM, Fanger MW. Monoclonal antibodies that bind to distinct epitopes on hFcγRI are able to trigger receptor function. J Immunol 1989; **143**:1650–5.

26 Looney RJ, Abraham GN, Anderson CL. Human monocytes and U937 bear two distinct Fc receptors for IgG. J Immunol 1986; **136**:1641–7.

27 Greenman J, Tutt AL, Georoe AJT, Pulford KAF, Stevenson GT, Glennie JJ. Characterization of a new monoclonal anti-FcγRII antibody, AT10, and its incorporation into a bispecific $F(ab')_2$ derivative for recruitment of cytotoxic effectors. Mol Immunol 1991; **28**:1243–54.

28 Brakenhoff JPJ, Hart M, de Groot ER, di Padova F, Aarden LA.

Structure-function analysis of human IL-6. J Immunol 1990; **145**:561–8.

29 Fletcher Starnes Jr H, Pearce MK, Tewari A, Yim JH, Zou JC, Abrams JS. Anti-IL-6 monoclonal antibodies protect against lethal *Escherichia coli* infection and lethal tumor necrosis factor-α challenge in mice. J Immunol 1990; **145**:4185–91.

30 Allen JM, Seed B. Isolation and expression of functional high affinity Fc receptor complementary DNAs. Science 1989; **243**: 378–81.

31 Stuart SG, Trounstine ML, Vaux DJT *et al.* Isolation and expression of cDNA clones encoding a human receptor for IgG. J Exp Med 1987; **166**:1668–84.

32 Peltz GA, Trounstine ML, Moore KW. Cloned and expressed human Fc receptor for IgG mediates anti-CD3-dependent lymphoproliferation. J Immunol 1988; **141**:1891–6.

33 Warmerdam PAM, van de Winkel JGJ, Gosselin EJ, Capel PJA. Molecular basis for a polymorphism of human Fcγ receptor II (CD32). J Exp Med 1990; **172**:19–25.

33a Warmerdam PAM, van den Herik-Oudijk IE, Parren PWHI, Westerdaal NAC, van de Winkel JGJ, Capel PJA. Interaction of a human FcγRIIb1 (CD32) isoform with murine and human IgG subclasses. Int Immunol (in press).

34 Tuijnman WB, Capel PJA, van de Winkel JGJ. Human low affinity IgG receptor FcγRIIa (CD32) introduced into mouse fibroblasts mediate phagocytosis of sensitized erythrocytes. Blood 1992; **79**:1651–6.

35 Brooks DG, Qiu WQ, Luster AD, Ravetch JV. Structure and expression of human IgG FcRII (CD32). Functional heterogeneity is encoded by the alternative spliced products of multiple genes. J Exp Med 1989; **170**:1369–85.

36 Helle M, Boeije L, Aarden L. Functional discrimination between interleukin 6 and interleukin 1. Eur J Immunol 1988; **18**:1535–40.

37 Koolwijk P, van de Winkel JGJ, Pfefferkorn LC, Jacobs CWM, Otten I, Spierenburg GT, Bast BJEG. Induction of intracellular Ca^{2+} mobilization and cytotoxicity by hybrid mouse monoclonal antibodies. J Immunol 1991; **147**:595–602.

38 Boros P, Odin JA, Muryoi T, Masur SK, Bona C, Unkeless JC. IgM anti-FcγR autoantibodies trigger neutrophil degranulation. J Exp Med 1991; **173**:1473–82.

39 Salmon JE, Brogie L, Edberg JC, Kimberley RP. Fcγ receptor III induces actin polymerization in human neutrophils and primes phagocytosis mediated by Fcγ receptor II. J Immunol 1991; **146**:997–1004.

40 Kishimoto T. The biology of interleukin-6. Blood 1989; **74**:1–10.

41 Van Damme J, Cayphas S, Opdenakker G, Billiau A, van Snick J. Interleukin-1 and poly(rI).poly(rC) induce proliferation of a hybridoma growth factor by human fibroblasts. Eur J Immunol 1987; **17**:1–7.

42 Debets JMH, van de Winkel JGJ, Ceuppens JL, Dieteren IEM, Buurman WA. Cross-linking of both FcγRI and FcγRII induces secretion of tumor necrosis factor by human monocytes, requiring high affinity Fc–FcγR interactions. J Immunol 1990; **144**:1304–10.

43 Parlevliet KJ, Jonker M, ten Berge RJM *et al.* Anti-CD3 murine monoclonal isotype switch variants tested for toxicity and immunologic monitoring in four chimpanzees. Transplantation 1990; **50**:889–92.

44 Chatenoud L, Ferran C, Reuter A. Systemic reaction to the anti-T-cell monoclonal antibody OKT3 in relation to serum levels of tumor necrosis factor and interferon-alpha. N Engl J Med 1989; **320**: 1420–1.

45 Abramowicz D, Schandene L, Goldman M. Release of tumor necrosis factor, interleukin-2 and gamma-interferon in serum after injection of OKT3 monoclonal antibody in kidney transplant recipients. Transplantation 1989; **47**:606–8.